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MUTATION INDUCTION IN HUMAN LYMPHOID CELLS BY ENERGETIC HEAVY IONS

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ABSTRACT

One of the concerns for extended space flight outside the magnetosphere is exposure to galactic cosmic radiation. In the series of studies presented herein, the mutagenic effectiveness of high energy heavy ions is examined using human B-lymphoblastoid cells across an LET range from 32keV/μm to 190 keV/μm. Mutations were scored for an autosomal locus, thymidine kinase (tk), and for an X-linked locus, hypoxanthine phosphoribosyltransferase (hprt). For each of the radiations studied, the autosomal locus is more sensitive to mutation induction than is the X-linked locus. When mutational yields are expressed in terms of particle fluence, the two loci respond quite differently across the range of LET. The action cross section for mutation induction peaks at 61 keV/μm for the tk locus and then declines for particles of higher LET, including Fe ions. For the hprt locus, the action cross section for mutation is maximal at 95 keV/μm but is relatively constant across the range from 61 keV/μm to 190 keV/μm. The yields of hprt-deficient mutants obtained after HZE exposure to TK6 lymphoblasts may be compared directly with published data on the induction of hprt-deficient mutants in human neonatal fibroblasts exposed to similar ions. The action cross section for induction of hprt-deficient mutants by energetic Fe ions is more than 10-fold lower for lymphoblastoid cells than for fibroblasts.

INTRODUCTION

The radiation environment in extramagnetospheric space is dominated by galactic cosmic radiation (GCR). It is comprised primarily of energetic protons and helium ions, but contains a small percentage of high energy heavy ions (HZE). It has been suggested that iron is the most important of the HZE particles because of its relative contribution to the galactic cosmic ray dose and its high linear energy transfer /1/. Risk estimates for extended manned missions in interplanetary space require assessment of the genotoxicity of energetic iron particles as well as primary particles of lower Z and secondary particles associated with fragmentation of iron particles as they pass through shielding. While direct assessment of the carcinogenic risk of HZE exposure is not possible in humans, it is possible to determine the risks for cell killing and for somatic mutation using a variety of human cell lines or strains of different tissue origin. Limited data are available on the cytotoxic and mutagenic effectiveness of these HZE particles in human fibroblasts /2/. These studies have focused on the induction of mutations at a single X-linked locus. In the series of studies described herein, the mutagenic effectiveness of HZE particles with LET's ranging from 32-190 keV/μm was assessed for two genetic loci, hprt and tk, in the human B-lymphoblastoid cell line, TK6. These loci were chosen as they are representative of the majority of human genes. The hprt gene spans 44 kb, is located on the X chromosome, /3,4/ and is hemizygous in this male-derived cell line. The tk gene spans 12.9 kb and is located on chromosome 17 /5,6/. It is heterozygous in TK6 cells by virtue of a point mutation which inactivates one of the alleles /7/.

METHODS

Cell Culture and Mutation Assays

TK6 lymphoblasts were grown in suspension culture in RPMI-1640 medium + 10% horse serum and 1% penicillin-streptomycin (GIBCO Laboratories, Grand Island, NY) at 37 degrees C in a humidified 5% CO_2 atmosphere. Cell density throughout an experiment was maintained at 4 x 10^5 cells/ml to 1 x 10^6 cells/ml by daily dilution. Cytotoxicity and mutation assays was performed as previously described /8/. Four to seven days prior to irradiation, cells were incubated for two days in normal growth medium supplemented with CHAT (10 μm deoxycytidine, 200 μm hypoxanthine, 0.2 μm aminopterin, and 17.5 μm thymidine) and for a further two days in normal growth medium supplemented with THC (CHAT without

aminopterin). Replicate flasks were irradiated for each exposure point in a given experiment to ensure that a minimum of 10^8 cells were exposed as a single culture. Immediately following the exposures, an aliquot of cells was removed for low density plating in 96 well microtiter dishes to determine the cytotoxicity of the treatment. The remainder of the culture was subcultivated in standard growth medium for a period of 3 days (tk locus) or 6-7 days (hprt locus) to permit complete expression of the mutant phenotype. Cells were seeded for mutation assays into 96-well microtiter dishes at a density of 40,000 cells/well in medium containing trifluorothymidine (TFT, Sigma Chemical Co., final concentration 2.0 μg/ml) to select for tk-deficient mutants. An aliquot of each culture was seeded at a density of one cell/well in non-selective medium to determine the cloning efficiency. After 11 days of growth, the fraction of mutants with a normal cell generation time (tk-ng) was calculated as previously described /9/. Dishes were refed with TFT and incubated a further 7 days, at which time slowly growing tk-deficient mutants (tk-sg) were scored. The total number of tk-deficient mutants per treatment is the sum of the yield of tk-ng and tk-sg mutants. The protocol for selection of hprt-deficient mutants was similar to the protocol used for the selection of tk-ng mutants with the exception that 6-thioguanine (6TG, Sigma Chemical Co., final concentration 0.5μg/ml) was used to select for hprt-deficient mutants.

Mutation and cytotoxicity data were obtained for a minimum of three independent experiments for each particle beam chosen. The data were fitted to the following equations using the method of least squares:

$$S/S_0 = e^{-D/D_0}, \qquad M = k_1 D$$

$$S/S_0 = e^{-\alpha\phi}, \qquad M = k_2 \phi$$

Where S/S_0 is the surviving fraction (%), M is the induced mutation fraction (%), D is the dose in cGy, and ϕ is the fluence (particles/cm^2). The parameter D_0 is 1/terminal slope of the survival curve and is in units of cGy. The inactivation cross section (μm^2) is defined as 16 x LET (keV/μm) x $1/D_0$. The action cross section for mutation induction is defined as $k_2 \times 10^6$. RBE for mutation induction was calculated for each locus as the ratio of the slopes k_1 (particle beam)/ k_1 (100 kVp X-rays).

Irradiation Conditions

X-ray exposures. X-irradiations were performed with a Phillips 100 kVp X-ray generator at a dose rate of 29.6 cGy/minute. Dosimetry was performed using a Victoreen ionization chamber.

Heavy ion exposures. Cells were irradiated with graded doses of ^{20}Ne, ^{28}Si, ^{40}Ar, or ^{56}Fe ions (Table I) at the BEVALAC heavy ion accelerator at Lawrence Berkeley Laboratory. Cells were exposed in the plateau region of the Bragg ionization curve for each of the beams used. A minimum of lead scatterer was utilized to spread the beam uniformly over a T-25 flask. Replicate flasks containing 5×10^7 cells in suspension culture were placed in the beam line. Dosimetry was performed using standard methods, and required coincident dose estimates from two nitrogen gas-filled ion chambers and one thimble-sized EG&G ion chamber along the beam path.

RESULTS

The results for cell killing for the series of beams from Ne 425 MeV/amu (LET = 32 keV/μm) to Fe 600 MeV/amu (LET = 190 keV/μm) are summarized in Table I. When the data are analyzed in terms of absorbed dose, Si ions (456 MeV/amu, LET=61 keV μm) were the most effective for cell killing. When the data are analyzed in terms of particle fluence, a single Fe ion (600 MeV/amu, LET=190 keV/μm) is the most effective for cell killing.

Table I

Summary of Beams Used and Results for Cell Killing

Radiation Type	LET (keV/μm)	D_0 (cGy)	Inactivation Cross Section(μm2)
Ne 425 MeV/amu	32	48	10.67
Si 670 MeV/amu	50	47	16.91
Si 456 Mev/amu	61	28	34.86
Si 330 MeV/amu	80	38	33.33
Ar 470 MeV/amu	95	34	44.71
Fe 600 Mev/amu	190	51	59.60

The results of recent mutation experiments using 600 MeV/amu Fe ions are shown graphically in Figures 1 and 2.

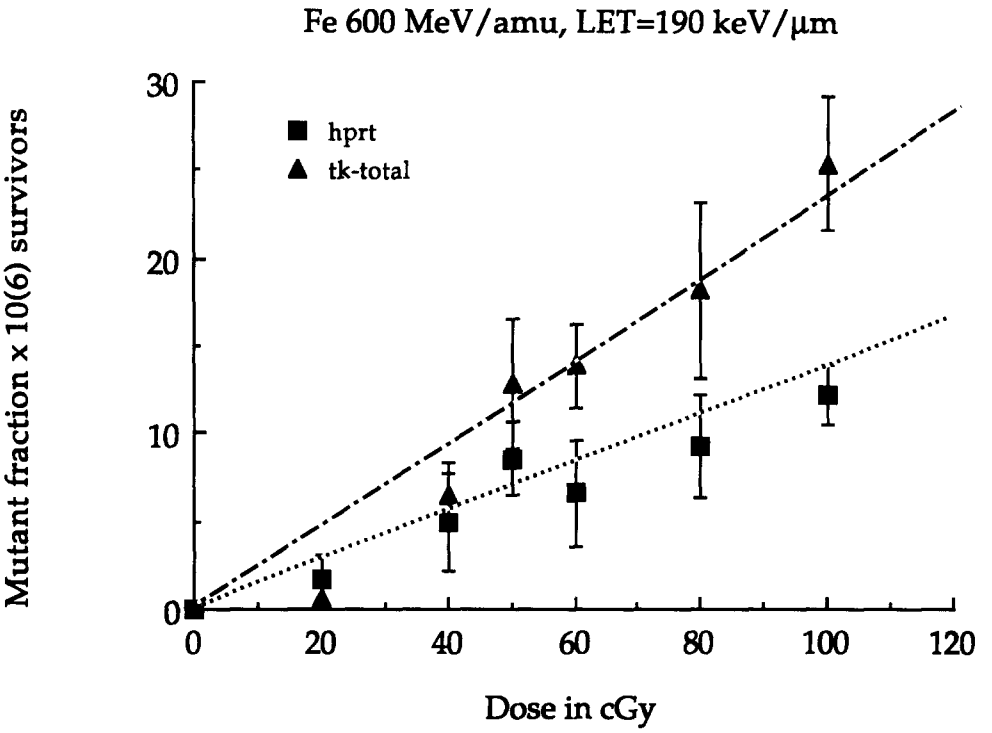

Fig. 1. Induction of hprt-deficient and tk-deficient mutants in human TK6 lymphoblasts by Fe ions (600 MeV/amu, LET=190 keV/μm) as a function of dose.

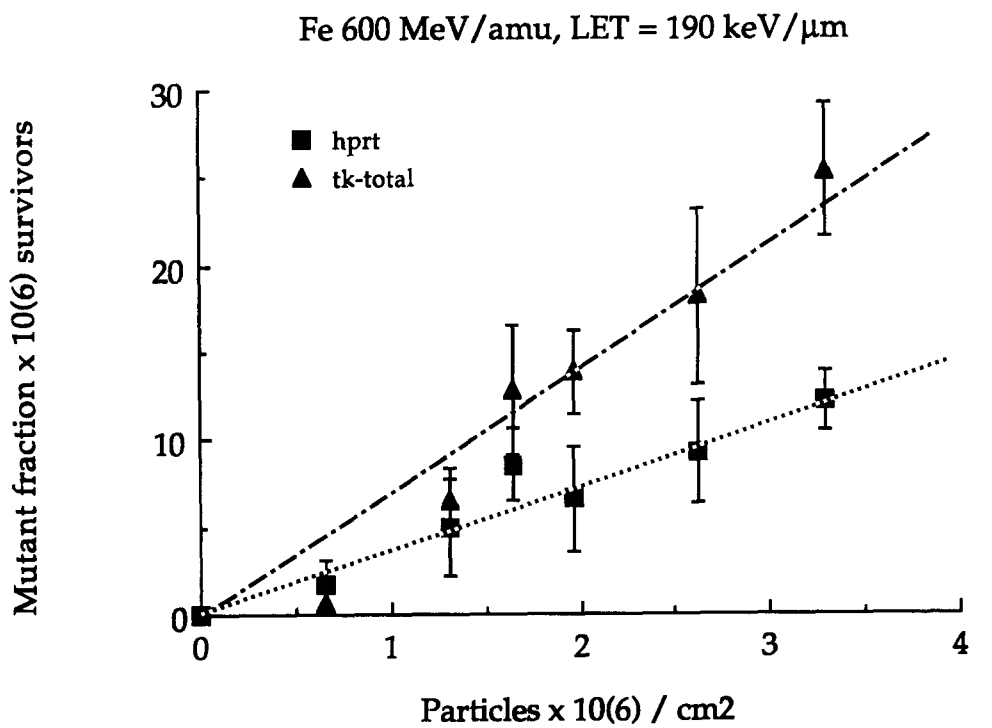

Fig. 2. Induction of hprt-deficient and tk-deficient mutants in human TK6 lymphoblasts by Fe ions (600 MeV/amu, LET = 190 keV/μm) as a function of particle fluence.

The dose range covered represents only a few particle traversals per cell nucleus (maximum dose = fewer than 3 particles per cell nucleus on average). The tk locus is more susceptible to mutation induction than is the hprt locus. These data are compared with the data obtained for the remaining particle beams (Tables II and III). When mutation yields are expressed in terms of absorbed dose, 456 MeV/amu Si ions were the most biologically effective beam for the induction of mutations at each locus (Table II).

Table II

Summary of Hprt and Tk Mutation Data as a Function of Dose

Type of Radiation	LET (keV/μm)	Locus	Induced Mutant Fraction x 10(-7)/cGy	RBE
100 kVp X-ray	1-2	hprt	0.6 ± 0.1	1.0
		tk-total	3.0 ± 0.3	1.0
Ne 425 MeV/amu	32	hprt	2.5 ± 0.1	4.2
		tk-total	4.5 ± 0.2	1.3
Si 670 MeV/amu	50	hprt	2.1 ± 0.1	3.5
		tk-total	4.7 ± 0.3	1.6
Si 456 MeV/amu	61	hprt	4.2 ± 0.4	7.0
		tk-total	16.1 ± 0.9	5.4
Si 330 MeV/amu	80	hprt	4.3 ± 0.3	5.6
		tk-total	8.2 ± 0.5	2.7
Ar 470 MeV/amu	95	hprt	3.0 ± 0.3	5.0
		tk-total	6.5 ± 0.5	2.2
Fe 600 MeV/amu	190	hprt	1.2 ± 0.1	2.0
		tk-total	2.3 ± 0.3	0.8

Of particular note is the observation that the Fe ion beam is less efficient at producing mutations at the tk locus than are 100 kVp X-rays when the data are described in terms of absorbed dose. The same is not true for the hprt locus. For the same absorbed dose, the Fe ion beam is twice as efficient as X-rays for the production of hprt-deficient mutants.

Another way to evaluate these results is to consider the relative mutagenic effectiveness of a single particle of a given type. The action cross-sections for mutation induction are presented in Table III.

Table III

Summary of Mutation Data as a Function of Fluence

Radiation Type	LET (keV/μm)	Locus	Action Cross Section ($\times 10^{-4}$ μm^2)
Ne 425 MeV/amu	32	hprt	1.27
		tk-total	2.31
Si 670 MeV/amu	50	hprt	1.67
		tk-total	3.76
Si 456 MeV/amu	61	hprt	4.09
		tk-total	15.8
Si 330 MeV/amu	80	hprt	3.35
		tk-total	10.49
Ar 470 MeV/amu	95	hprt	4.60
		tk-total	9.84
Fe 600 MeV/amu	190	hprt	3.73
		tk-total	7.0

For the hprt locus, there is a rise in the mutational efficiency of a single particle with increasing LET. The most efficient particle for mutation is the Ar beam with an LET of 95 keV/μm. Iron ions of 190 keV/μm are slightly less efficient at producing hprt-deficient mutants in TK6 cells. The pattern is somewhat different for the tk locus. The maximal mutagenic efficiency is achieved with Si ions with an intermediate LET (61 keV/μm), and there is a steep decline in biological effectiveness for particles with increasing LET.

DISCUSSION

In the course of a three year mission to Mars, the most likely exposure of human cells to HZE particles will come as single particle traversals. It has been estimated that 8.4% of all cells in the body will be traversed by single ions with Z between 26 and 28 at some point in time /10/. Exposure to high energy protons will occur much more frequently and single cells are likely to be traversed every few days. These estimates were based on the CREME radiation environment which is presently undergoing revision. We know very little about the susceptibility of human cells of different tissue origin to mutation following exposure to low fluences of HZE particles and even less about the differential susceptibility of different loci within the human genome. The range of inter-individual differences in susceptibility to mutation following exposure to HZE particles is not yet known.

There are both qualitative and quantitative differences mutation yields as a function of LET for the hprt locus and the tk locus in TK6 lymphoblasts. Figure 3 summarizes the results for both loci as a function of absorbed dose.

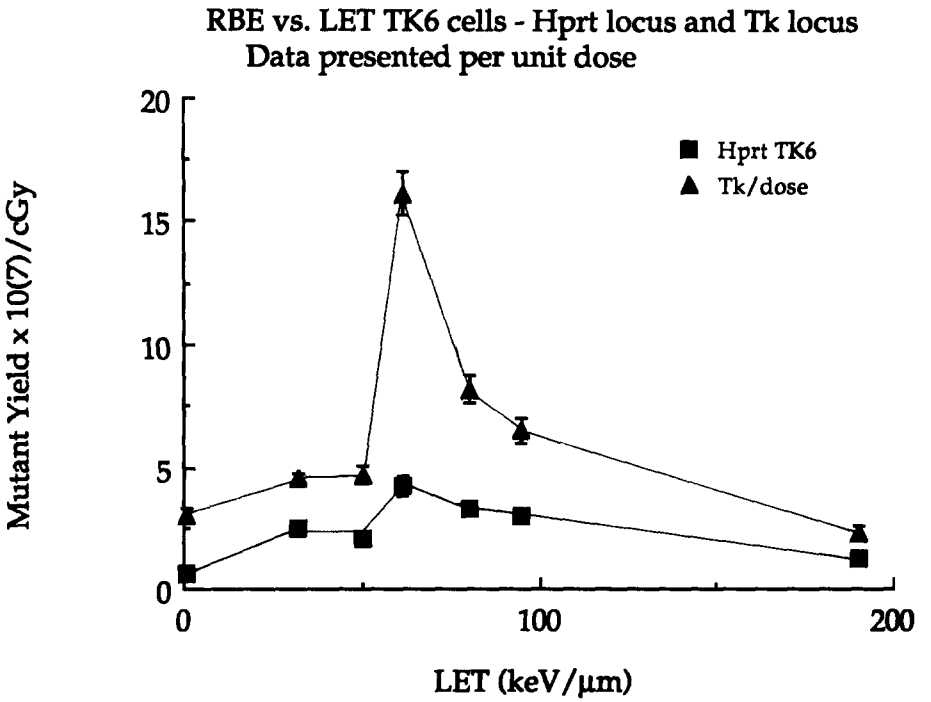

Fig. 3. Comparative yields of hprt-deficient and tk-deficient mutants by HZE particles in human TK6 lymphoblasts as a function of LET and absorbed dose.

Although the tk locus is generally more responsive to mutation induction across the range of LET's, the differences become minimized for particles with LET greater than 61 keV/μm. This is due primarily to the failure to recover slowly growing tk-deficient mutants at higher LET's. Figure 4 summarizes the results obtained for both loci as a function of particle fluence.

Mutations are more readily recovered for the autosomal tk locus than for the X- linked hprt locus, even though the hprt locus spans a larger piece of DNA. The mutational efficiency of single particles with LET's greater than 61 keV/μm is relatively constant for the hprt locus, while it declines rapidly for the induction of tk-deficient mutants. The molecular basis for this qualitative differences is under investigation.

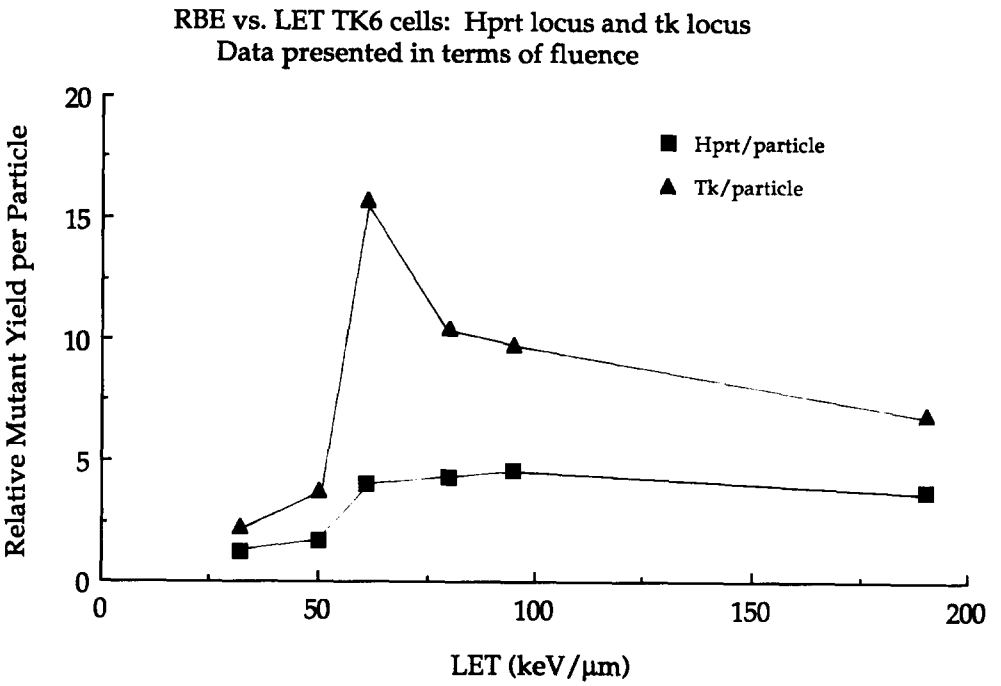

Fig. 4 Comparative yields of hprt-deficient and tk-deficient mutants by HZE particles in human TK6 lymphoblasts as a function of LET and particle fluence.

The results presented herein on mutation in human B-lymphoblastoid cells are best compared with results obtained for human fibroblasts /2/. If we compare the action cross sections for the induction of hprt-deficient mutants, human fibroblasts are much more likely to be mutated than are the TK6 lymphoblasts. A nearly direct comparison can be made only for the beam of Fe ions (600 MeV/amu, LET = 190-200 keV/μm depending on the amount of lead scatterer used) and the hprt gene is 15 times more likely to be mutated in fibroblasts than it is in the lymphoid cells. Inactivation cross sections do not differ significantly for the two cell types, although they are somewhat larger in the fibroblasts (92.0 μm^2 for Fe 600 MeV/amu) than for the lymphoblasts (59.6 μm^2 for Fe 600 MeV/amu). This is not unexpected, since the average geometric cross section of the nuclear area of the human skin fibroblast is 220 μm^2 while the corresponding area of the nucleus of TK6 lymphoblasts 86.6 μm^2. For all of the particle beams used in the present studies, the inactivation cross section was smaller than the average geometric cross section of the TK6 nucleus, confirming that multiple traversals would be required to induce a lethal lesion in all cells at risk. We can be reasonably certain, therefore, that immunoglobulin producing lymphocytes are likely to survive the most likely exposure to isolated HZE particles, if we are willing to neglect other influences the space environment may have on lymphocyte turnover and cytokine production.

For particles of intermediate LET, it is necessary to compare the results obtained in human lymphoblastoid cells with high energy heavy ions with data obtained in fibroblasts using low energy light ions /11,12,13/. While the nature of the particle tracks will differ significantly /14/, the trend in the data confirms that the TK6 cells are substantially less sensitive across a broad range of LET to mutation at the hprt locus than human fibroblasts obtained from several different individuals. One possibility is that this reflects a difference in the susceptibility to mutation that is specific to cell lineage. Another possibility is that there may be something unusual in the processes of cellular enzymatic repair of initial DNA damage arising after exposure to of TK6 cells to ionizing radiation. The result is that less of the initial damage is "fixed" as a heritable genetic alteration. There have been several studies wherein the susceptibility to radiation-induced mutation has been evaluated in paired repair-deficient and repair-competent cell lines. No difference was observed in the induction of hprt-deficient mutants by either X-rays or alpha particles in wild type CHO K1 cells or their double-strand break repair-deficient counterpart, xrs-5 /15/. The xrs-5 cells were more susceptible than the CHO K1 cells to radiation-induced cell killing and the production of chromosome aberrations. The double-strand break repair-deficient murine lymphoblast L5178Y-S1(LY-S1) was

somewhat less susceptible to mutation at the hprt locus than was the repair-competent L5178Y-R16 (LY-R16) /16/. The hprt mutant yield was reduced in the LY-S1 cells by less than a factor of 2. When susceptibility to mutation was evaluated at the tk locus in the LY-S1 and LYR-16 cells, the double strand break repair-deficient LY-S1 cells were about three-fold more susceptible to mutation induction than the LY-R1 cell line -- in direct conflict with the trend observed for another locus in the same cells.

The TK6 cell line was derived from WIL-2 cells after several rounds of selection /17/. It has been reported that TK6 cells are more sensitive to cell killing and are less sensitive to mutation than two other subclones of WIL-2, WTK-1 and WTB-B. The TK6 subclone appears to be two-fold less sensitive to mutation induction at the hprt locus, and 25-100 fold less sensitive to mutation induction at the tk locus than is seen in the other WIL-2 subclones /18/. The underlying basis for this difference in sensitivity is unknown. Nevertheless, the data obtained at the hprt locus would suggest that the major difference in the susceptibility of human fibroblasts and human B-lymphoid cells to mutation induction by HZE particles is a result of differences in cell lineage. Further studies are needed using other lymphoid cells in order to determine the range of sensitivities among individuals.

The range of variation in the susceptibility to mutation induction by ionizing radiations may be larger for autosomal genes than for an X-linked genes in males, due to allelic heterogeneity on paired chromosomes. It has been shown that the spectrum of radiation-induced mutations, and HZE-induced mutations in particular, is dominated by large deletions /19/. These deletions may extend over millions of basepairs of DNA , and the ability to detect the largest deletions may be limited by the presence of a heterozygous essential gene at some distance down the chromosome from the target locus. For a gene which resides on a single copy chromosome, such as the X chromosome in male cells, the distance to an essential gene would be the same in all individuals. The variation in susceptibility amongst individuals would be controlled by other factors. The limits on the ability to recover large deletions involving an autosomal gene would depend on the number of essential genes found along the same chromosome, and the number of functional alleles of those genes in a cells from each individual. Differences in susceptibility of the two copies of the tk gene to large deletion mutation have been observed in closely related cells /20/ and in all likelihood reflect differences in the functional status of remote alleles of one or more essential genes on the two copies of chromosome 17 in these cells.

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