A Review of Forty-Five Years Study of Hiroshima and Nagasaki Atomic Bomb Survivors

II. BIOLOGICAL EFFECTS

Current Summary of Lymphocyte Survival Study

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A recently developed dose-survival assay in vitro using human G0 T-lymphocytes from peripheral blood was employed to assess possible interindividual variation of cellular radiosensitivity. Currently lymphocytes from a total of 99 atomic bomb survivors were tested and D10, the X-ray dose required to kill 90% of the cells, was calculated for each test. The mean ± SD of D10 value was 3.35 ± 0.22 Gy for 61 survivors whose DS86 dose is below 0.004 Gy and for 38 survivors of DS86 dose above 1.5 Gy it was 3.31 ± 0.26 Gy. So far, the results do not show any evidence in support of the hypothesis of a selective elimination of a radiosensitive subcohort among the survivors exposed to high doses.

INTRODUCTION

The epidemiologic data for the atomic bomb survivors in Hiroshima and Nagasaki serve as an important source of information for the assessment of risks of the various late health effects induced in human population1). However, to fully understand the relationship between exposure to radiation and subsequent possible effects, it is critical to determine whether the atomic bomb survivor's radiosensitivity is representative of a normal population, and, if not, to characterize the extent of their departure from normality. Since many people perished from acute radiation injury, it is possible that the surviving heavily exposed population preferentially lost some relatively radiosensitive individuals compared with those exposed to low dose or nonexposed population. That is, the group of heavily exposed survivors may be on average relatively less radiosensitive when compared to the nonexposed group. If this dose-related "healthy survivors" effect were to exist, then radiation-related risks to human populations may actually be higher than currently thought.

In the present study, in vitro radiosensitivity of peripheral blood lymphocytes was compared between the heavily exposed survivors and 0 Gy controls, under the assumption that genetic heterogeneity is equally expressed in bone marrow cells (major target of human death caused by radiation exposure of below several gray) and in lymphocytes (current measurement).
MATERIALS AND METHODS

The lymphocyte colony assay has been described in detail\textsuperscript{2}. In brief, peripheral blood lymphocytes were seeded in 96-well round bottom microplate with feeder cells consisting of allogeneic lymphocytes and lymphoblastoid cells (B-cell line) irradiated with 50 Gy of X-rays prior to use. Subsequently, test lymphocytes were irradiated with 0 to 5 Gy of X-rays, incubated for 2 weeks and the presence or absence of lymphocyte colony was determined for each well microscopically. The fraction of wells without a colony was used to calculate cloning efficiency (CE) assuming a Poisson distribution of colonies since one cannot distinguish whether each colony is derived from a single clonogenic cell or from 2 or more cells. Namely, $CE = -\ln\left(\text{fraction of wells without colony}/\text{average number of cells seeded per well}\right)$. The survival data of each experiment were first fitted to a linear quadratic equation, $-\ln CE = aD + bD^2 + c$ using an unweighted least squares method ($a$, $b$ and $c$ are constants, $D$ is X-ray dose in Gy) to obtain fitted CE at zero dose, viz., $e^c$. Subsequently, the $D_{10}$ value, the dose required to reduce the fitted CE at zero dose to one-tenth, viz., $e^{c/10}$, was calculated.

X-irradiation was performed by Shimadzu WSI-250S machine at 220 KVp, 8mA with a 0.5 mm Al and 0.3 mm Cu filter and a dose rate of about 0.25 Gy/min. Total dose was measured for all the experiments by a Victoreen 500 dosimeter calibrated at the Research Institute for Nuclear Medicine and Biology of Hiroshima University.

RESULTS

The mean $\pm$ SD of $D_{10}$ was $3.35 \pm 0.22$ Gy after single tests of 61 survivors with DS86 dose of below $0.004$ Gy, $3.31 \pm 0.26$ Gy after single tests of 38 survivors with DS86 dose of greater than $1.5$ Gy. Fourteen repeat tests of lymphocytes from a single donor produced a $D_{10}$ of $3.42 \pm 0.26$ Gy, quite similar to the above mentioned values, although the total number of tests is comparatively small. These results did not show any evidence that the average radiosensitivity differs between the two groups of the atomic bomb survivors.

DISCUSSION

In our previous studies, it has been found that lymphocytes bearing CD4 or CD8 surface antigen mainly form colonies under the present culture conditions\textsuperscript{3}. Subsequent study revealed that dose-survival curves of sorted CD4\textsuperscript{+}, CD8\textsuperscript{+} or unsorted lymphocytes were very similar\textsuperscript{4}. Therefore, although the subset frequency of peripheral blood lymphocytes varies among individuals, use of unsorted lymphocytes does not introduce biases to the dose-survival results caused by differential subset radiosensitivity as far as in vitro colony assay is concerned.

Results of the present study showed a mean $D_{10}$ value of $3.33 \pm 0.24$ Gy for a total of 99 individuals. The coefficient of variation (CV) is 7.2%. Compared with the CV obtained after repeated tests of lymphocytes from a single donor, viz., $CV = 7.6\%$ (14 repeat tests in the present study) and $5.7\%$ (28 repeat tests in our previous study)\textsuperscript{5}, it appears that intrinsic interindividual variation in cellular radiosensitivity is very small, if it exists at all, and is quite difficult to identify.
individuals of altered cellular radiosensitivity.

One may suspect that the mode of lymphocyte death is different from that of other types of cells and hence interindividual variation was masked. However, it has recently been shown that G0 lymphocytes from ataxia telangiectasia patients are definitively more radiosensitive than those from apparently normal people as has been previously demonstrated with skin fibroblasts. Therefore, there appears no reasons to suppose peculiar mode of lymphocyte death under the current experimental conditions in vitro. Our previous study using matched samples of skin fibroblasts and G0 lymphocytes from same individuals showed that the CV for the mean D10 value was about twice as great in the log phase fibroblast assay than in the G0 lymphocyte assay. The results strongly suggest that the G0 lymphocyte assay includes less confounding factors, such as possible selective biases in the fibroblast population during establishment of fibroblast strains, cell cycle distribution, passage number etc.

In conclusion, current results for the in vitro dose-survival assay using G0 lymphocytes revealed very little interindividual variation, if any, in cellular radiosensitivity and so far no evidence was observed in support of the conjecture that genetically radiosensitive individuals had been selected against, among the atomic bomb survivors who received more than 1.5 Gy. The study is still in progress and a more extensive report will be prepared in near future.

REFERENCES


