HERITABLE CELL CYCLE DISTURBANCES AND LATE RECOVERY IN X-IRRADIATED MURINE LYMPHOMA L5178Y-S CELL POPULATIONS IN VITRO

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In the last decade the view has been expressed by several authors (1-4) that ageing of the normal mammalian cells is connected with the prolongation of the mitotic cycle. At the same time it has been shown that ionizing radiation can in vitro (5-10) and in vivo (11,12) induce in mammalian cells, besides the lethal effects, also some heritable damages which retard the cell cycle traverse for considerable number of cell generations.

In this paper we will outline results of our studies on the heritable damages including cell cycle disturbances and late recovery phenomena in X-irradiated murine lymphoma L5178Y-S cells. This cellular model system in the form of a suspension culture presents an object particularly suitable for long term studies on growth disturbances (9).

EXPERIMENTAL

The technique of in vitro cultivation of the radiosensitive strain L5178Y-S of murine leukaemic lymphoblasts (13-15) has been described previously (8,9,17). Mean doubling times (T_D) for controls varied in individual experiments from 10 to 14 hr.

Cell population densities were determined microscopically using a Burker haemocytometer.

The cultures were irradiated with 200 kV X-rays filtered with 0.5 mm Cu, HVL 1 mm Cu at a dose rate of 345 rads per min (9,17).
Viability of the cell populations was determined by nigrosine staining (17).

Cloning of the cells in agar-supplemented medium (18) and isolation of the slowly growing cell sublines were described in (9,16,19,20).

 Autoradiographic examination of the cell cycle was presented by Beer et al (21). The cell generation time ($T_D$) and durations of $G_1$, $S$ and $G_2$ phases ($T_{G1}$, $T_S$ and $T_{G2}$, respectively) were determined according to Mendelsohn and Takahashi (22). Frequency distribution of the $G_2$ phase duration was determined according to Stanners and Till (23).

RESULTS AND DISCUSSION

In a majority of cases irradiation of L518Y-S cells with doses of 25 to 600 rads of X-rays causes growth and viability disturbances which persist for periods corresponding to more than one hundred control cell generations, as it can be seen in Figure 1. More comprehensive description of these changes can be found in Beer et al. (8) and Beer (9).

The retardation of growth is partially caused by the presence of the slowly-growing clones of the cells. This has been proven by cloning followed by observations of growth of the sublines isolated from non-irradiated cell cultures and from the cell populations irradiated with 300 rads of X-rays. In Figure 2 it can be seen that the sublines with $T_D$ almost double as compared to that of the controls derived from single cells present in the cultures which survived irradiation. Viability of the cloned sublines with $T_D$ not exceeding 18 hr was normal (95%). Somewhat lower viabilities were observed in the slower sublines. The decrease of viability could account, however, only for a very minor part of the growth retardation. Even the highest observed content of dead cells which amounted to 17% could add a little more than 2 hr to the control $T_D$ value of 10.8 hr (25). In fact, the sublines with 17% of dead cells grew with $T_D$ of 20 hr or more.

The cell cycle of control L5178Y-S line and of several slowly growing sublines was analyzed autoradiographically. It was found that although this analysis in the case of control cells was relatively easy, the percentage of labelled metaphases for the slowly growing sublines often were so scattered that setting up of the curve for conventional analysis was impossible. In these cases highly irregular relationships could be predicted. Results suitable for analysis of the cell cycle were obtained in two cases