increase in DSBs over unirradiated control and was chosen for the repair experiments (Fig.1B and C).

Of the cell lines examined, LY-R and GM00736A showed a substantial decrease in the level of DNA damage 30 min after irradiation. In the case of LY-S, GM00717C, and Reh cells no apparent difference was seen between the initial (time "0") DSBs and the DSBs 30 min after irradiation (Fig.1B and C). DSBs repair deficiency in LY-S cells and homozygous *ataxia telangiectasia* cells was in agreement with the previous reports [11,12]. A defect in DSBs repair after X-irradiation in human Reh cell line has, to our knowledge, not yet been described.

The results presented here are a pilot study. To enable studying DSBs repair after biologically relevant doses, sensitivity of the method has to be further improved. This can be achieved by adjusting electrophoretic conditions and enhancing the DNA detection efficiency. There is a possibility to use a new DNA dye with fluorescence much exceeding that of ethidium bromide (e.g. GelStar, FMC Bioproducts, USA) - for direct staining of DNA in the gel, or DNA transfer from the gel to a nylon membrane (Southern blotting) and subsequent identification of the bands with a specific digoxygenine-labelled DNA probe and immunoenzymatic, signal amplificating reaction.

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CELL CYCLE ARRESTS AND APOPTOSIS IN X-IRRADIATED DIFFERENTIALLY RADIOSENSITIVE L5178Y SUBLINES

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We examined the cell cycle arrest, apoptosis and expression of BAX and BCL2 proteins in two L5178Y (LY) murine lymphoma sublines, LY-R and LY-S. They differ in radiation sensitivity slower double strand break (DSB) rejoining is seen in LY-S (radiation sensitive). Both sublines carry a mutation in codon 170 of p53 [1]. LY-R cells do not constitutively express BCL2, whereas both sublines show high BAX content. The levels of these proteins do not change after irradiation. There is no G1 arrest and a difference in G2 arrest, short in LY-R cells and long in LY-S cells. Radiation induces delayed apoptosis to a greater extent in LY-S than in LY-R cells; it can be seen 24 h after irradiation (2 Gy) of LY-S cells, with a maximum at 48 h; LY-R cells need 5 Gy and 72 h to show marked apoptosis (identified by the flow cytometry TUNEL method (Fig.1A) and the "ladder" pattern of DNA electrophoresis (Fig.1B)). The effect revealed by both methods was considerably more pronounced in LY-S cells than in LY-R cells.

In the TUNEL method apoptotic LY-S cells were seen 24 h after irradiation and the maximum was reached at 48 h. The electrophoretic pattern for LY-S irradiated with 5 Gy was closer to a smear than to ladder pattern at 72 h after irradiation (Fig.1B), indicating late necrosis. Apoptotic LY-R cells could be found at later time intervals and

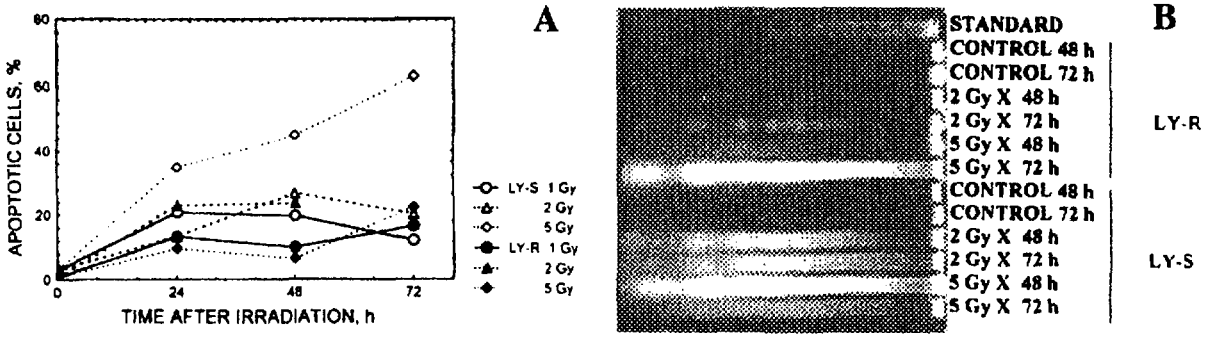

Fig.1. Apoptosis in X-irradiated LY cells. A - % of apoptotic cells determined by flow cytometry-TUNEL in cell cultures X-irradiated with 1, 2 or 5 Gy; B - patterns of DNA electrophoresis (standard - DNA from apoptotic, heat-treated LY-S cells).

higher doses. This could be clearly seen also on dot plots of TUNEL-stained cells (not shown).

The delayed apoptosis is much more frequent in LY-S than in LY-R cells, even at a roughly comparable survival level (2 Gy for LY-S cells, surviving fraction (SF)=ca 0.04; 5 Gy for LY-R cells, SF=ca 0.02. However, it is impossible to predict the cell tendency to undergo apoptosis judging from the expression of p53, bcl-2, bax, c-myc and bcl-x2 [2]; clearly, more information is needed on the functioning and cross-talks of signalling pathways. Also, apoptosis is not always a reliable indicator of radiation sensitivity, as recently reviewed by Hendry and West [3].

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DO LOW DOSES OF IONIZING RADIATION INDUCE CHROMOSOMAL REPAIR ACTIVITY? SCREENING HUMAN T CELL EXTRACTS MICROINJECTED INTO MOUSE EMBRYOS

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The adaptive response of human lymphocytes to ionizing radiation has been proposed to involve a chromosomal repair activity [1]. Several reports have appeared in support of low dose induction of new proteins in human lymphocytes. Youngblom et al. [2] have found that the presence of the protein synthesis inhibitor cycloheximide between the adapting and challenging doses prevented the detection of an adaptive response. Wolff et al. [3] have reported on the identification of new proteins induced by low doses of X-rays.

The observations of low dose induced gene products in human lymphocytes, while intriguing, remain associations, and not identification of molecular factors responsible for the adaptive response. We chose alternate approaches, by searching for a chromosomal repair activity present in extracts from low dose exposed human T cells. The activity was screened in mouse embryos that, themselves, fail to exhibit an adaptive response and thus provided a background free from this variable [4]. Embryos were microinjected with the whole cell extracts from the same human T cell population that exhibited an adaptive response for micronucleus induction. Chromatid aberrations were scored in microinjected embryos challenged with 0.5 Gy of X-rays in G_2 phase.

Mouse embryos microinjected with the whole cell extracts from low dose exposed Jurkat cells had fewer aberrations than those microinjected with control extracts (Fig.). These extracts came from the same population of Jurkat cell that exhibited an adaptive response to micronucleus induction. It is tempting to conclude that some component(s) of the extracts was responsible for the reduced cytogenetic damage in both human and mouse cells. However, the reduction in 0.5 Gy-induced aberrations in the mouse embryos was not at the level observed for micronuclei in the Jurkat cells (25 vs

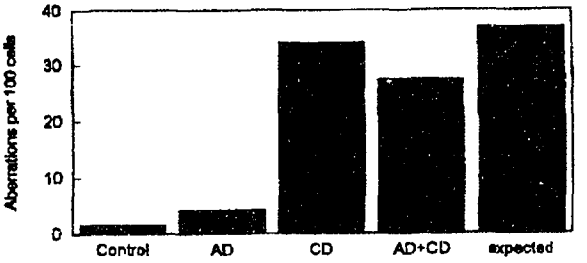

Fig. Chromosomal aberrations in embryos microinjeced with extracts from Jurkat cells. AD - adapting dose, CD - challenging dose.

40%), nor was the reduction in aberrations statistically significant. Thus, this result strongly indicates that the adaptive response in Jurkat cells is either not due to the inducible chromosomal repair activity or that the activity responsible is not common to the human and mouse cell types tested.

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