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DNA REPAIR IN ADAPTED HUMAN LYMPHOCYTES

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In our previous work [1] we demonstrated that the development of adaptive response in human lymphocytes requires the presence of calcium ions in the medium at the time of applying the adapting dose. This result was interpreted in terms of alarm signal hypothesis [2]. It could be expected that adaptation to the challenge dose is accompanied by a higher DNA repair rate and that intervention at the level of intracellular signal transduction would abolish the development of the adaptive response both at the cellular level (revealed by micronuclei frequency) and at the level of DNA repair.

To examine this assumption, the following experiments were carried out. Lymphocytes from 4 human (non-smoker) donors received an adapting dose of 0.01 Gy of X rays 6 h after stimulation with phytohaemagglutinin and a challenge dose of 1.5 Gy 10 h later. The adaptive response in three donors and its lack in the fourth donor was identified by the micronuclei frequency estimated after cytochalasin B treatment and cell harvest at 72 h (Fig.1).

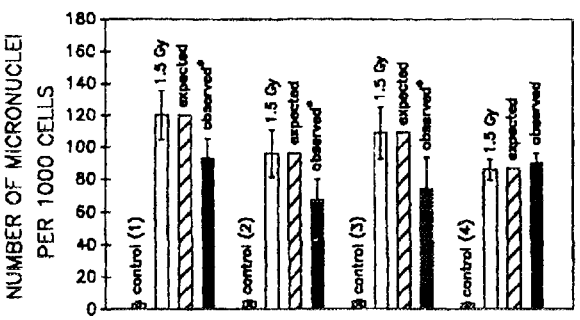

Fig.1. Comparison of the adaptive response induced in human lymphocytes with 1 cGy X-rays. The end-point analyzed was the frequency of micronuclei (5000 cells scored per experimental point). The first 3 donors show a statistically significant (Student's t-test, $P<0.001$) difference from the expected micronuclei frequency. Donor 4 has shown a lack of adaptive response.

Initial DNA damage and its repair rate were measured with the "comet" assay immediately after giving the challenge dose (on ice). Although in some experiments lower initial DNA damage and higher repair rate were observed in adapted, as compared to non-adapted lymphocytes (Fig.2), this was not a rule. On the other hand, lymphocytes from the donor lacking adaptive response (as esti-

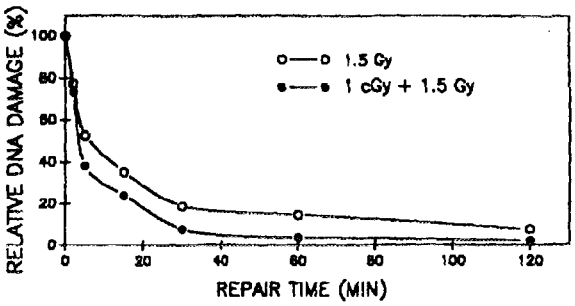

Fig.2. DNA repair rate (per cent damage remaining) in human lymphocytes after 1.5 Gy X-irradiation with or without 1 cGy pre-exposure.

mated by the micronuclei test) repaired DNA damage at the same rate, irrespectively of irradiation with the adapting dose or sham-irradiation. These results are consistent with those of Wójcik et al. (in press), who observed a discrepancy between adaptation estimated from chromosomal aberration frequency and from DNA repair rate measured by the "comet" assay. Moreover, inhibitors of signal transduction, such as staurosporine, TMB-8, and anti-CD38 antibody, applied together with the adapting dose, prevent the development of the adaptive response, as identified by the micronuclei test. Nevertheless, these inhibitors do not significantly affect the rate of DNA repair in lymphocytes subjected to such combined (inhibitor+adapting dose) treatment. So, there is a discrepancy in the manifestation of the adaptive response at the cellular and molecular level. The higher rate of DNA repair, sometimes observed in the adapted cells, as compared to the non-adapted ones, does not seem to be a constant feature of adaptation.

References

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INTERDEPENDENCE OF INITIAL DNA DAMAGE, ITS REPAIR AND SENSITIVITY TO TOPOISOMERASE I POISON, CAMPTOTHECIN

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Two L5178Y (LY) murine lymphoma cell sublines, LY-R, resistant, and LY-S, sensitive to X-radiation, display inverse cross-sensitivity to camptothecin (CPT): LY-R cells are more susceptible to this specific topoisomerase I inhibitor than LY-S cells. After 1 h incubation with CPT, the doses that inhibit growth by 50% (ID_{50}) at 48 hrs incubation are 0.54 μM for LY-R cells and 1.25 μM for LY-S cells. Initial numbers of DNA-protein crosslinks (DPCs), measured at this level of growth inhibition, are two-fold higher in LY-R (5.6 Gray-equivalents) than in LY-S cells (3.1 Gray-equivalents), which corresponds well with the greater in vitro sensitivity of Topo I from LY-R cells to CPT [1, 2]. Converse-