Repair of ionizing radiation induced DNA damage in human lymphocytes

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ABSTRACT

Phytohemagglutinin stimulated human lymphocytes exhibit a 20 fold increase in DNA repair synthesis following ionizing radiation damage compared to the level of repair in unstimulated cells. The peak of repair synthesis coincides with that for DNA replication. Stimulated lymphocytes provide a relatively simple assay for ionizing radiation repair defects.

INTRODUCTION

The radiobiology of lymphocytes has been extensively studied and distinct populations of lymphocytes have been identified by their differential sensitivity to ionizing radiation (1). Several reports indicate that B lymphocytes are more radiosensitive than T lymphocytes (2-5), but it is well recognized that resistance to ionizing radiation varies within subpopulations of both cell types (6-9). Mitogen stimulated T cells are more resistant to radiation than quiescent cells (10, 11). It is suggested that this increased protection is due to the induction of DNA repair enzymes and one report which shows a doubling of repair synthesis after stimulation of lymphocytes for 18 hr with phytohemagglutinin supports this contention (12).

Several genetic disorders are known which show increased sensitivity to radiation and defective repair of damage to DNA (13-15). In most cases these disorders also have accompanying abnormalities and disturbances of the immune system (16). This suggests the possibility that some of the steps involved in DNA repair are also involved in the development or maturation of lymphocytes. Accordingly a detailed study of the repair capabilities of lymphocytes is of importance in further understanding of these disorders. In this report we have

examined ionizing radiation induced DNA repair in quiescent and phytohemagglutinin stimulated lymphocytes at daily intervals after addition of mitogen. The results obtained indicate that a dramatic increase in repair capacity occurs in transformed lymphocytes which is in keeping with the observed greater radioresistance of these cells. Lymphocytes represent a useful source of cells to examine repair defects because of the rapidity with which the assays can be carried out.

MATERIALS AND METHODS

Lymphocyte isolation. Peripheral blood lymphocytes from volunteers were separated by sedimentation on methyl cellulose-hypaque according to Moss and Pope (17), and were plated out in media (F15, Gibco + 10% fetal calf serum) + 1% phytohemagglutinin (v/v, Gibco), where indicated.

DNA replication. Synthesis of DNA was measured at 1 day intervals after addition of phytohemagglutinin. Cells were incubated for 4 hr with [^3H]thymidine (Radiochemical Centre, Amersham; 25 Ci/mmol, 5 $\mu\text{Ci/ml}$). Cells were then centrifuged at 350 g for 10 min, washed once in saline-EDTA solution (18) and recentrifuged. The cell pellet was finally resuspended in saline-EDTA and a sample was removed to determine cell counts. Saline-EDTA allowed dispersion of cells which were found to clump in the presence of phytohemagglutinin, and thus facilitated counting. An equal volume of cold 10% trichloroacetic acid was added to the cells, which were kept on ice for 10 min. Filtration was carried out using Whatman GF/C filters, washing with 5% trichloroacetic acid, then ethanol. The filters were dried and counted using a toluene scintillator.

DNA repair. Repair synthesis was measured in the presence of 10 mM hydroxyurea (19), added 30 min before irradiation; this reduced DNA replication to about 1% of the uninhibited value. Cells were irradiated in a Gamma Cell 220 (Atomic Energy of Canada, Ltd) at a dose rate of 6.5 Krads/min.

[^3H]Thymidine (5 $\mu\text{Ci/ml}$) was added immediately prior to irradiation since the irradiation process occupied periods up to 10 min, during which time some repair was already occurring. Control samples were treated in the same way, without irradiation. After a total of 4 hr incubation with label, cells

were washed and filtered as described above. Repair synthesis was calculated as the difference between [^3H]thymidine incorporation (in the presence of hydroxyurea) in irradiated cells and in unirradiated cells. This approximately corrects for the contribution of incorporation as replication in the irradiated cells (19).

Density gradient centrifugation. Cesium chloride density gradient analysis was used to determine that the incorporation of radiolabel in the presence of hydroxyurea in irradiated cells over and above that observed in unirradiated cells was indeed in the form of repair synthesis. Bromodeoxyuridine was added to cells for 1 hr to prevent end-to-end labelling (20). After 30 min incubation in bromodeoxyuridine (5 $\mu\text{g/ml}$) hydroxyurea (10 mM) was added for a further 30 min. Cells were then irradiated in the presence of [^3H]thymidine (10 $\mu\text{Ci/ml}$) and incubated for 5 hr. DNA was prepared by the procedure of Smith & Hanawalt (21) using proteinase K. This extract in 0.1M K_2HPO_4 was adjusted to pH 12.5 with NaOH, 1.76 g/ml with cesium chloride and centrifugation carried out in a 50 Ti rotor of a Beckmann L265B centrifuge at 35,000 rpm for 60 hr. Gradients were pumped out using a Gilson peristaltic pump, then refractive indices and cpm were determined in each fraction.

RESULTS

Figure 1 shows the radiation induced incorporation of [^3H]thymidine into peripheral blood lymphocytes, in the presence of hydroxyurea, 120 hr after addition of phytohemagglutinin. Residual replication occurring in the presence of hydroxyurea in unirradiated cells has been subtracted. Repair synthesis increased with dose up to 35 Krads and leveled off at higher levels of radiation. When incorporation of label into lymphocytes with increasing radiation dose was determined in the absence of phytohemagglutinin a reduction of some twenty-fold was observed. This pattern of incorporation is consistent with increased capacity for repair synthesis resulting from phytohemagglutinin stimulation, i.e. phytohemagglutinin induces a DNA repair system that is at a low level or absent in quiescent lymphocytes.

Exposure of human lymphocytes to an ionizing radiation dose

Fig. 1 (Left): DNA repair in human lymphocytes with increasing dose of ionizing radiation. Unstimulated cells (●), cells stimulated with phytohemagglutinin 5 days prior to irradiation (■). The results presented represent the mean values of eleven experiments.

Fig. 2 (Right): Time course of DNA repair in phytohemagglutinin stimulated lymphocytes irradiated with 65 Krad. Cells were incubated with phytohemagglutinin for 5 days. Unstimulated cells (●), cells stimulated with phytohemagglutinin (■).

of 65 Krad gave rise to a rapid initial incorporation of [3 H]thymidine over the first hour after irradiation (Fig. 2). At later times a decrease in the rate of incorporation was observed but net incorporation continued to increase for several hours (Fig. 2). Again, in the absence of phytohemagglutinin, a very low level of radiation induced repair was obtained. To this point it was assumed that incorporation of [3 H]thymidine with radiation dose was indeed as repair. However it has been demonstrated that phytohemagglutinin stimulated human lymphocytes selectively replicate limited portions of their genome and give rise to a DNA intermediate which is later excreted from the cells (22). Accordingly, to rule out the possibility that radiation was inducing selective replication we used cesium chloride density gradient analysis to distinguish between DNA replication and repair. The results in Fig. 3 show that for irradiated cells a peak, indicative of repair synthesis, appears in the light density region (1.74 g/ml), in addition to a heavy peak of DNA (1.82 g/ml) representing residual replication.

Fig. 3. Equilibrium alkaline cesium chloride density gradient of DNA from unirradiated cells (----), and from cells exposed to 65 Krads of gamma radiation (—) in the presence of hydroxyurea (10^{-2} M).

Having optimized the conditions with respect to radiation dose (Fig. 1) and time of incubation (Fig. 2), the extent of repair occurring at daily intervals after addition of phytohemagglutinin was determined. No repair synthesis was detectable for the first 48 hr after addition of phytohemagglutinin, but a low level was detected after 72 hr (Fig. 4). A marked increase in repair synthesis took place

Fig. 4. Time course of DNA replication and repair of ionizing radiation (65 Krads) damage to DNA at daily intervals after addition of phytohemagglutinin. DNA replication (●), DNA repair (■). This pattern of replication and repair was observed in lymphocytes from 8 donors with little variation.

after 72 hr, reaching a peak at 96-120 hr, followed by a decrease with time. This peak of repair synthesis was coincident with the peak of DNA replication induced by phytohemagglutinin. In the absence of phytohemagglutinin repair synthesis occurred at a minimal level.

DISCUSSION

These data demonstrate that phytohemagglutinin stimulated human lymphocytes show a high level of DNA repair synthesis following ionizing radiation exposure when compared to unstimulated cells. An earlier report (12) showed that after 18 hr in the presence of phytohemagglutinin a 2-fold increase in repair synthesis occurred after exposure of lymphocytes to ionizing radiation. From our experiments it is evident that repair synthesis is low in lymphocytes for up to 72 hr after phytohemagglutinin addition, but at 96-120 hr a 20-fold rise occurs over the value in unstimulated cells. This increase in DNA repair synthesis could explain the higher radioresistance of mitogen activated lymphocytes compared to resting cells (10, 11).

The greater capacity of stimulated lymphocytes to repair damage in DNA has also been described for UV irradiated cells (23) and cells exposed to alkylating agents (24). The occurrence of a peak of ionizing radiation induced repair synthesis several days after addition of phytohemagglutinin is analogous to the findings with alkylating agents (24). On the other hand exposure of human lymphocytes to UV is reported to give two peaks of repair synthesis (23). The first peak, at 3-4 days after phytohemagglutinin, corresponds to those above and with the peak of DNA replication, while the second peak occurs three days later. In all three cases, the overall pattern of repair synthesis is suggestive of a repair system which is integrally associated with DNA replication, or is dependent on enzymes induced in a co-ordinate fashion. Indeed, there is evidence that induction of enzymes occurs as DNA replication increases (25-27); a number of these enzymes has been implicated in DNA repair (28).

Our results, which demonstrate a large increase in the repair capacity of phytohemagglutinin stimulated lymphocytes relative to unstimulated cells, indicate that activated

lymphocytes provide a potentially useful assay system for genetic disorders characterized by increased radiosensitivity (29). The relative rapidity of measurement of DNA repair in lymphocytes provides a simpler alternative to fibroblasts for assay of ionizing radiation repair defects.

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