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APPLICATION OF RADIATION-INDUCED APOPTOSIS IN RADIATION ONCOLOGY AND RADIATION PROTECTION

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A rapid assay of the ability of lymphocytes to respond to radiation-induced damage is presented. Age and genetic dependence of radiation response have been quantified. The assay is sensitive to low doses of radiation. Its ability to assess the cytotoxic response of blood capillaries to radiation has been evaluated.

For assays of radiation response, blood is considered the tissue of choice because of ease of collection in a standardised, convenient manner. Following exposure to ionising radiation, lymphocytes respond via apoptosis, i.e., physiological cell death. Apoptosis was quantified flow cytometrically by determining the percent of cells with an apparently reduced DNA content. This could always be clearly discriminated in both CD4 and CD8 T-lymphocytes. Apoptosis increased with radiation dose. Apoptotic cells display the smaller, 2–2.5 fold, cell size and slightly increased, 1.1–1.2 fold, granularity characteristic of physiological cell death. These cells, but not vital cells, stain positively for DNA strand breaks using a commercially available terminal deoxynucleotidyl transferase kit and positively for phosphatidylserine using a commercially available annexin kit; both features are typical of apoptotic cells. Neither do they exclude vital stains; indicating dead cells with permeable membranes. Fluorescence and electron microscopy of apoptotic lymphocytes reveal whole cells with a single, large, apoptotic chromatin mass instead of a normal nucleus.

45 donors were studied to investigate features of radiation-induced apoptosis. Radiosensitivity was different between the two cell-types; CD8 T-lymphocytes usually displayed more radiation-induced apoptosis than CD4 T-lymphocytes. No correlations between spontaneous (0 Gy) and radiation-induced apoptosis, between gender and radiation-induced apoptosis levels, or between the time prior to exposure and radiation-induced apoptosis levels were observed. However, a decrease in radiation-induced apoptosis with age was observed (0.5% less radiation-induced apoptosis per year). We examined blood from five young donors for enhanced cytotoxic response. They all displayed increased radiation-induced apoptosis, confirming the trend observed.

In collaboration with the Children's hospital in Zurich, blood from two children with genetic diseases and requiring radiation treatment was also studied. Low levels of radiation-induced apoptosis in the CD4 T-lymphocytes from an immunodeficiency, chromosome fragility, facial anomaly (ICF) patient, and the CD4 and CD8 T-lymphocytes from an ataxia telangiectasia (AT) patient were observed. Since the ICF patient had profound CD4 lymphopenia (90–100 per µl as compared to 600–2500 per µl in healthy age-matched blood), selection for CD4 T-lymphocytes deficient in apoptosis may be occurring in her blood. Apoptotic response has not been investigated in the ICF cases previously reported in the literature. AT is associated with a well-documented abnormal radiation-response. A number of studies have demonstrated reduced apoptosis in AT lymphocytes 24-h following radiation exposure, a finding we confirmed. Reduced apoptosis is attributed to a cell's inability to respond to radiation-induced damage. This inability to mount a response to radiation damage results in enhanced levels of tissue-toxic cell necrosis. In collaboration with the radiation oncology clinics in Geneve and Lausanne, blood from adult patients displaying acute hypersensitive responses to radiation therapy were also observed to be deficient in radiation-induced apoptosis.

The assay is sensitive to low doses of radiation. At doses below 1 Gy, levels of radiation-induced apoptosis increase for up to 5 days after irradiation. Greatest sensitivity is observed 4 days post- irradiation. The dose response relationship is linear with a slope of 8 % per 0.1 Gy. Radiation-induced apoptosis is significantly higher than background controls levels even after 0.05 Gy exposures. The assay is suitable for various applications as a radiation accident dosimeter and in radiation worker screening.

The versatility of the assay has promoted parallel studies with non-lymphoid tissues of interest. Blood vasculature is essential for growth of tumours. Its selective destruction causes tumour necrosis and inhibition of neoplasia which reduces tumour progression. The endothelial cells which build the blood capillary network are derived from normal healthy tissues and are not subject to genetic instability, therefore, they provide a constant homogeneous target for therapy. We have initiated studies with human umbilical vein endothelial cells and bovine aortic endothelial cells. However, rather than apoptosis, these cells tend to undergo radiation-induced mitotic cell death accompanied by rapid subsequent disintegration. This process requires entry into mitosis and as this is not synchronised, unless a means to inhibit cell disintegration is found, quantification of cytotoxicity in these cells will either require monitoring of individual cell fates or be indirect via measurement of cell clonogenicity.