CHANGES OF THE LYMPHOCYTE POPULATIONS AND THEIR ROLE AS A BIOLOGICAL INDICATOR FOR IONIZING RADIATION

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1 Introduction

It is known for some time (see UNSCEAR Report 1972) that increased doses of ionizing radiation have a negative influence on natural body resistance. In recent years detailed work was done on the effect of ionizing radiation on the immune system, also in man. For these examinations mainly lymphocytes were used, since they frequently proliferate during their development to effector cells. For this reason, their sensitivity to radiation is very high. For many of the radiation-induced effects examined, dose-response curves could be developed.

Within the scope of a research project, supported by the Radiation Protection Programme of the Commission of the European Communities (Contract BIO-C-518-82-D), it is to be clarified to which extent effects of ionizing radiation on the lymphocytes can be used for a "biological dosimetry". It is the aim of the project to find an additional method for the approved and well established, but personnel- and time-consuming method of chromosome analysis. Initially, the relative and absolute number of lymphocytes and their subpopulations in the peripheral blood and the number of immunoglobulins produced by the lymphocytes are the main objective of the examinations.

2 Methods

For differentiating the lymphocytes, a new method developed by "Bio-Rad" is used and modified for our purposes. From heparinized whole blood the mononuclear cells (monocytes and lymphocytes) are isolated via density-gradient separation with Ficoll-Paque. Consecutively, the cells are incubated with so-called immunobeads. Due to rosette formation with the differently coloured beads, the subpopulations of the lymphocytes can be differentiated. The monocytes are identified by phagocytosis of the immunobeads.

At first, the blood of normal controls is examined in order to both optimize the method and obtain comparative values for the absolute and relative number of lymphocyte subpopulations in normal controls. Also the blood of normal controls and mononuclear cells, isolated from this blood, are irradiated in vitro with different doses. After this, the distribution of the lymphocyte subpopulations is evaluated, according to the method described above. The objective of this study is to yield dose-response curves for the change of the proportional and absolute values of the lymphocyte subpopulations.

Another important item is the assessment of the viability of the lymphocytes, the number of the immunoglobulin producing cells

and the amount of immunoglobulin production by the lymphocytes after prior irradiation.

For this purpose, either whole blood, isolated mononuclear cells or isolated lymphocytes are irradiated in vitro. Then the mononuclear cells or lymphocytes which were initially isolated or subsequently separated from whole blood are cultured. The cells are stimulated in culture with "pokeweed mitogen" to proliferate and mature to immunoglobulin producing cells. In some of the tests, cells are repeatedly removed during culturing time in order to determine their vitality by a dye exclusion test with trypan blue. In further experiments, at the end of the culturing time the number of immunoglobulin producing cells is determined by immunofluorescence and the amount of the produced immunoglobulins is found by the ELISA test and nephelometry. In place of the lymphocyte subpopulations or mononuclear cells in their physiological distribution, these can also be cultivated and examined in defined numerical proportions, as described above.

The objective of the work is also in this case to develop dose-response curves for the change of the given parameters, depending on the dose of ionizing radiation applied in each case.

3 Preliminary Results and Discussion

The above described method for differentiating mononuclear cells is a relatively simple process that requires little time. As compared to the currently used method, where B-cells are determined by immunofluorescence and T-cells in an individual process by a rosette test, the technique presented here has the advantage that monocytes and subpopulations of lymphocytes can be counted at the same time. In contrast to the established method for biological dosimetry, the chromosome analysis, our method is less personnel- and time-consuming and requires less experienced personnel. The disadvantage being that the absolute values and the proportional distribution of lymphocyte subpopulations may be quite different in physiology from individual to individual, so that the method might not be as sensitive and accurate as chromosome analysis in the lower dose range. Besides this, the values may vary when influenced by different pharmaceutical drugs.

An extension of the examinations to the blood of radiation therapy patients is intended to clarify to which extent the dose-response curves, after in vitro irradiation, are applying to in vivo conditions. Furthermore, it is to be examined whether the influence of other easily to be assessed parameters may increase the sensitivity and accuracy of the method.

In the method where cells are cultured in order to determine vitality and immunoglobulin production, a larger sensitivity is to be expected for lower dose values, since the radiation effect is increased due to cell cultivation. In this connection it has to be clarified whether a suitable combination of cells from the individual subpopulations or the use of other parameters might limit the biological variability and thus enables a greater accuracy of dose assessment. Here, too, the examinations shall be extended to the blood of radiation therapy patients to find out

whether the dose-response curves after in vitro irradiation can also be applied when irradiated in vivo. It is, however, of disadvantage that on account of the time required for cultivating cells, results are yielded not earlier than after several days, similarly to the analysis of structural chromosome aberrations. On the other hand, the time required as, e.g., for determining the immunoglobulin production at the end of cell culturing is relatively short and may be further reduced by automation.