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RADIATION SENSITIVITY OF LYMPHOCYTES FROM HUMAN BLOOD AND FROM THE THORACIC DUCT

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The role of the lymphocyte in various immunologic reactions has been greatly clarified during the last ten years (GOWANS & MCGREGOR 1965, WEGELIUS et coll. 1970). The central role of the lymphocytes in cell mediated immunologic reactions such as transplantation immunity has led to attempts to control or reduce their activity by various methods. One way is to use ionizing radiation. Lymphocytes are quite sensitive to radiation, and irradiation has therefore been used in connection with organ transplantation to cause immunosuppression (ROSENGREN & SKÖLDBORN 1968). The irradiation is directed either towards a limited site in order to achieve local immunosuppression, or towards lymphoid tissues or peripheral blood lymphocytes to achieve a more generalized effect. Analysis of the lymphocyte function after irradiation provides methods for the evaluation of the immunosuppressive effect of therapy and might also under certain circumstances be used as a biologic radiation dosimeter (ILBERY et coll. 1971).

The present investigation was undertaken to evaluate the radiation sensitivity of blood and thoracic duct lymphocytes, and to investigate the possibility of using the lymphocyte function for evaluating the effects of extracorporeal irradiation of lymphatic cells.

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Material and Methods

Lymphocytes were collected from peripheral blood of 5 healthy subjects and by cannulation of the thoracic duct from 2 patients with rheumatoid arthritis. The blood lymphocytes were isolated and purified as described previously (LINDAHL-KIESSLING 1972), except that the carbonyl-iron treatment of the blood was omitted. The thoracic duct lymphocytes were purified by washing three times in culture medium.

The cells were irradiated at room temperature before isolation from blood or lymph, immediately after collection of the samples. A dose rate of 220 rad/min, 250 kV, 15 mA and a filtration of 1 mm Cu, was used.

Cell cultures were performed in triplicate by suspending 0.5 or 1.0×10^6 lymphocytes in 2 ml Eagle's minimum essential medium supplemented with l-glutamine and 10 % pooled, inactivated, human AB-serum, in 16 mm × 125 mm polycarbonate tubes. The cultures were incubated for 66 hours in a humidified 5 % CO_2 in air atmosphere; 18 hours before termination 1 μCi ^{125}I-deoxy-uridine was added. The cultures were terminated by washing the cells twice in ice-cold saline, and the activity was counted in a well-type scintillation counter.

Lymphocytes were activated by adding to the cultures doses of 0.5 to 10 μg/ml kidney bean leucoagglutinin (LA) purified as described by RÄSÄNEN et coll. (1973) and prepared at Medix Labs Ltd., Helsinki, Finland.

Details of the 2 patients will be reported elsewhere (EDGREN et coll. 1976). In brief, they were subjected to thoracic duct cannulation and the lymphocytes drained were irradiated with 2 800 rad and returned to the patients by intravenous infusion. Duct drainage was continued for 14 and 18 days, respectively, during which time 24 and 29 l of lymph were collected, irradiated and reinfused, except 2 l which were used for cell cultures and other investigations. The lymph contained a mean of 4.5×10^9 and 3×10^9 lymphocytes/l. Cell cultures were made from peripheral blood of the patients before drainage and 1 and 2 to 3 weeks after the beginning of the treatment.

Results and Discussion

The effect of irradiation of peripheral blood lymphocytes appears in Fig. 1. At 1 000 rad, at least a 50 per cent decrease was obtained in the ability of the lymphocytes to incorporate ^{125}I-deoxy-uridine after stimulation with LA in vitro. However, with higher doses a plateau was reached and the in vitro response of lymphocytes was not even abolished with 2 800 rad. This could be due either to a very resistant lymphocyte population or to a diminished incorporation of deoxy-uridine into all lymphocytes.

It is known that lymphocytes become either totally activated with lectins or not at all, never only partly activated, i.e. in a given single cell the response to stimulation is zero or maximum (HANDMAKER et coll. 1969). In order to resolve these alternatives, the response of irradiated lymphocytes to varying doses of LA was examined. If a

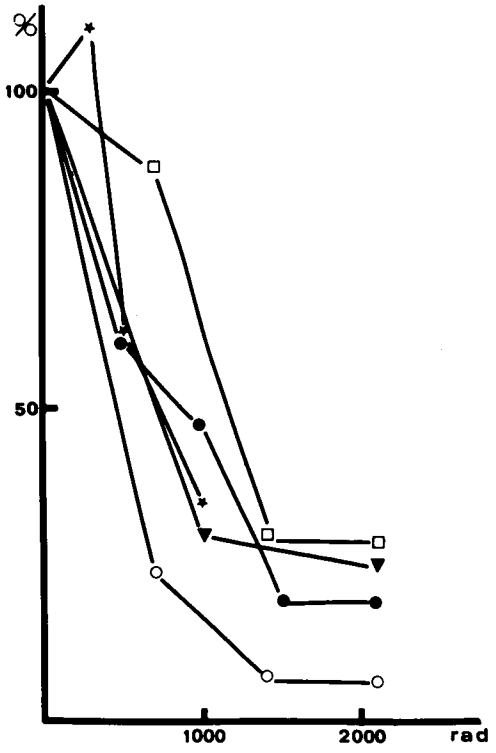

Fig. 1. Effect of irradiation on the function of peripheral blood lymphocytes from 5 subjects. Incorporation of ^{125}I-deoxy-uridine into DNA of lymphocytes stimulated with 10 μg/ml kidney bean LA. The incorporation into non-irradiated lymphocytes was taken as 100 per cent. Abscissa: radiation dose in rad. Ordinate: incorporation into lymphocytes (in per cent).

resistant lymphocyte population existed, a different dose response curve could be expected for these cells than for the whole population. On the other hand, if the incorporation of radiation activity was decreased in all cells after irradiation, similar dose response curves for treated and untreated cells would be expected. The results appear in Fig. 2. One cell population reacted to very low doses of LA in vitro. With increasing doses of LA more and more cells were activated. However, after irradiation with 2 800 rad, the cells activated by high doses of LA were eliminated and only the cells reacting to low doses remained. These cells appeared to be very resistant and the response was essentially similar in cells irradiated with 2 800 rad and in untreated cells. This clearly indicates that there is a lymphocyte population characterized by high sensitivity to stimulation by lectins and high resistance to radiation. The exact function and nature of this population is not known, but the reason for the low sensitivity to radiation might be the fact that before radiation certain metabolic reactions had occurred in these cells, reactions which are easily disturbed in other cells and which are required during lymphocyte activation in vitro. The possibility of the existance of what is called 'preactivated lymphocytes in blood' has previously been suggested. One feature of a pre-activated cell is that it has performed at least some RNA synthesis and that it is very sensitive to stimulation by lectins (WEBER et coll. 1974).

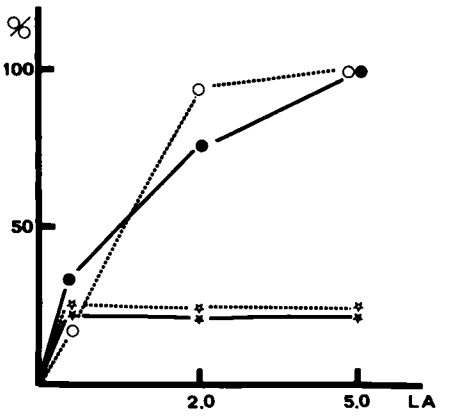

Fig. 2. Dose response (in per cent) of irradiated (2 800 rad) and non-irradiated thoracic duct lymphocytes from 2 subjects to different doses of kidney been LA (μg/ml). The two upper curves: non-irradiated cells; the lower curves: irradiated cells.

No significant alteration of the function of the lymphocytes from peripheral blood of the patients subjected to extracorporeal irradiation of thoracic duct lymphocytes was found. If the stimulation is expressed as an index obtained by dividing the counts of stimulated cultures with counts of unstimulated cultures, the mean of the results from two patients was: before extracorporeal irradiation 14 (range 11–16), after one week of thoracic duct drainage and irradiation 13 (range 11–16), and after the completion of thoracic duct drainage and irradiation, i.e. 2 to 3 weeks after the beginning of the treatment, 12 (range 8–17). This absence of significant alterations is most likely due to the rapid elimination of irradiated lymphocytes from peripheral blood. It has been shown that lymphocytes damaged by radiation remain in the peripheral blood for a mean of only 2 min (FIELD et coll. 1972). This makes it impossible to use peripheral lymphocytes as a measure of the effect of extracorporeal irradiation of blood or lymph, although it has been claimed in one report that this treatment causes a depression of peripheral lymphocyte activation (ROSENGREN et coll. 1968). The view that the irradiated lymphocytes are rapidly eliminated from peripheral blood is also supported by the fact that no irradiation-induced chromosomal aberrations were seen in the blood lymphocytes of these patients (EDGREN et coll.). In one of the patients, scanning of ^{51}Cr-labelled irradiated lymphocytes was performed and a definite uptake in the spleen was demonstrated, which indicates that the reticuloendothelial system eliminates the damaged cells from the circulation (EDGREN et coll., FIELD et coll.).

It may also be concluded that an analysis of lymphocyte activation after accidental total body irradiation is not suitable for evaluating the radiation dose, because the lymphocytes seem to react only at doses which are lethal and also because pre-irradiation controls are lacking. Thus the only reliable method for biologic dosimetry based on the use of peripheral blood cells is the time-consuming method employing chromosomal analysis (EVANS 1972).

SUMMARY

Peripheral blood and thoracic duct lymphocytes are sensitive to irradiation. However, a distinct population of resistant lymphocytes exists, which is characterized by reactivity to very low doses of lymphocyte-stimulating lectins in vitro. Blood lymphocytes are rapidly eliminated from the circulation after radiation damage. An analysis of the function of blood lymphocytes is thus of little or no use in assessing the therapeutic effect of extracorporeal irradiation. Lymphocyte activation must accordingly also be unsuitable for use as a biologic dosimeter after accidental total body irradiation.

ZUSAMMENFASSUNG

Das periphere Blut und die Lymphozyten des Ductus thoracicus sind gegenüber Bestrahlung empfindlich. Es liegt jedoch eine distinkte Population resistenter Lymphozyten vor, die sich durch ihre Reaktivität gegenüber sehr niedrigen Dosen von Lymphozyten-stimulierenden Lectinen in vitro auszeichnen. Blut Lymphozyten werden nach einer Schädigung durch Strahlung rasch aus der Zirkulation entfernt. Eine Analyse der Funktion von Blut Lymphozyten ist deshalb von geringem oder keinem Wert bei der Feststellung des therapeutischen Effekts der extracorporalen Bestrahlung. Die Aktivierung von Lymphozyten muss deshalb auch als biologisches Dosimeter nach accidentellen Gesamtkörperbestrahlung unanwendbar sein.

RÉSUMÉ

Les lymphocytes du sang périphérique et du canal thoracique sont sensibles à l'irradiation. Cependant il existe une population distincte de lymphocytes résistants qui est caractérisée par sa réactivité à de très faibles doses de lectines stimulant les lymphocytes in vitro. Les lymphocytes sanguins sont rapidement éliminés de la circulation après les lésions dues aux radiations. L'étude de la fonction des lymphocytes sanguins est donc de peu d'intérêt ou sans intérêt pour apprécier l'effet thérapeutique de l'irradiation extracorporéale. L'activation lymphocytaire doit donc aussi ne pas convenir comme dosimètre biologique après une irradiation accidentelle de tout le corps.

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