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A DIRECT EVIDENCE FOR THE EARLY MEMBRANE DESIALYLATION IN COBALT-IRRADIATED MOUSE LYMPHOCYTES

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Using the sensitive periodate-borotritide radioassay to quantify the membrane sialic acid amount, we show that the in vitro [^{60}Co]-irradiation of mouse splenocytes (10-50 Gy) significantly decrease their membrane sialic acid amount. The results show that the irradiation-induced desialylation is a very early phenomenon since the periodate oxidation is done immediately after the irradiation. A short incubation of cells at 25° C does not increase the extent of the desialylation. This membrane alteration might explain the rapid and drastic decrease in lymphocyte counts in mammals exposed in vivo to irradiation.

INTRODUCTION

Mammals exposed to ionizing radiations show a rapid and drastic decrease in their peripheral blood lymphocyte counts (1). When reinjected intravenously into syngeneic recipients, in vitro irradiated lymphocytes present an altered selective homing with an increased hepatotropism (2, 3). When reinjected, neuraminidase treated lymphocytes present a comparable modification in their redistribution (4). Since the irradiation of several cell types leads to a decrease in their electrophoretic mobility (5 - 10), as does a neuraminidase treatment (11), it is tempting to assess that irradiation induces a decrease in the membrane sialic acid amount. Using the extremely sensitive periodate-borotritide radioassay (12), we show here that the [^{60}Co]-irradiation of mouse splenocytes significantly decreases the membrane sialic acid amount.

MATERIAL AND METHODS

Cell suspensions and irradiation. Male Swiss C 17-DUPLAN mice breeded in the C.R.S.S.A. were used. Spleen cells were obtained in 0.15 M phosphate-buffered saline,

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pH 7.2. After washing, the cell concentration was adjusted to 10^8 cells per ml. Cells were irradiated at 4° C with a ^{60}Cobalt source at a dose rate of 4.5 Gy (450 rads) per min. Control cells were kept at 4° C.

Treatment of cells with sodium periodate and potassium borotritide. One volume of the cell suspension adjusted to 2 x 10^7 cells per ml was added to an equal volume of sodium periodate solution (5 mM), for 10 min at 4° C. Cells were then washed and the concentration was adjusted to 2 x 10^7 cells per ml. An equal volume (1.25 mCi) of potassium [^{3}H]-borohydride (36 Ci/mmole, CEA, Gif-sur-Yvette, France) was added. Reduction last 20 min at 20° C. Cells were finally washed with phosphate-buffered saline. In order to minimize the surface components shedding, all steps, but the borotritide reduction, were performed at 4° C.

Identification and quantification of sialic acid derivatives. Labelled lymphocytes were resuspended in 1 ml 0.1 N H_2SO_4 and hydrolyzed at 80° C for 60 min. Supernatants were then submitted to paper chromatography using Whatman 3 paper with butyl acetate/acetic acid/water, (3 : 2 : 1 by vol). Tritiated C_8-analog of N-acetyl neuraminic acid (N-AN 8) and C_7-analog (N-AN 7) prepared as described by Durand et al. (12) were used as standards. Radioactivity comigrating with N-AN 8 and N-AN 7 was quantified with the static radiochromatogram reader Chromelec (Numelec-Sein, le Mesnil St Denis, France). The radioactivity N-AN 8 plus N-AN 7 expressed per 10^6 cells from irradiated cells was compared with the controls'one.

RESULTS AND DISCUSSION

Fig. 1 shows the migration pattern of the radioactivity in hydrolysates of spleen lymphocytes treated with periodate and tritiated borohydride. Three peaks are essentially visualized. The first corresponds to non migrating compounds and partly originates from the tritiated borohydride. The second corresponds to N-AN 8 and the third to N-AN 7. Irradiated lymphocytes show a comparable pattern.

Fig. 2 shows that 50 Gy-irradiated lymphocytes immediately oxidized with periodate, present a significant decrease (34 %, $p < 0,02$) in their sialyl residues compared to control cells. In eight independent experiments, the periodate-sensitive sialic acid of 50 Gy-irradiated splenocytes represents 77,2 % $\pm$ 8 (SD) compared to control cells. 10 Gy-irradiated cells (Fig. 2) present a 23 % decrease in their periodate-sensitive sialic acid ($p < 0,05$) while 5 Gy-irradiated cells only present a 9 % decrease which is not significant. A short incubation (60 min, 25° C) of 50 Gy-irradiated cells before periodate oxidation does not induce a further decrease in the membrane sialic acid. In these conditions the periodate sensitive sialic acid of 50 Gy-irradiated splenocytes represents 75,9 % $\pm$ 15 (SD) compared to control cells (mean of six experiments).

The biphasic curve (Fig. 2) suggests that the desialylation concerns either a subpopulation of spleen lymphocytes or a subpopulation of cell surface glycoconju-

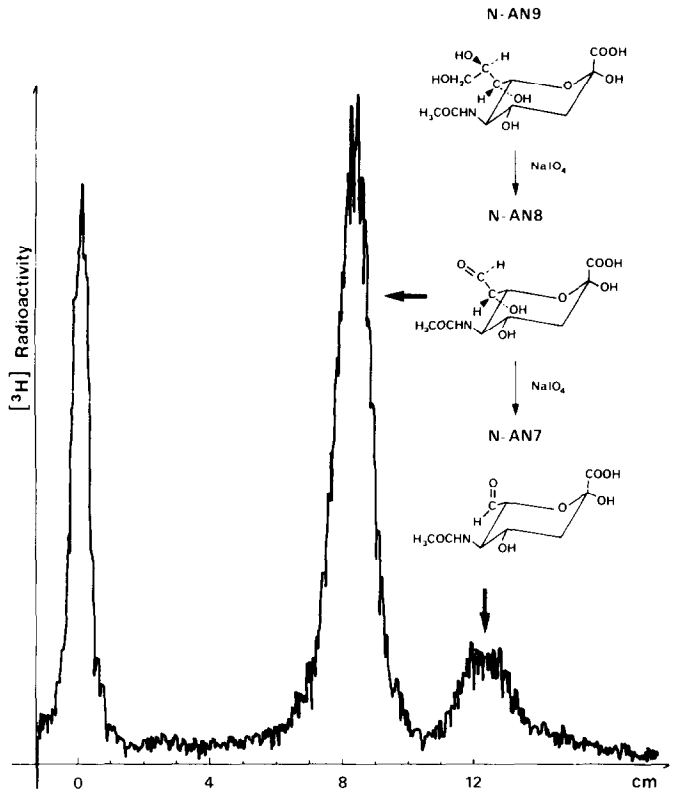

Figure 1 : Paper chromatography of acid hydrolysis products of mouse splenocytes treated with sodium periodate and tritiated borohydride. Peak migrating in 8 is the C_8-analog of N-acetyl neuraminic acid (N-AN 8) ; peak migrating in 12 is the C_7-analog (N-AN 7). The oxidation pattern of N-acetylneuraminic acid (N-AN 9) by periodate is represented.

gates. The first assumption would agree with the results of several teams showing that B cells are more radiosensitive than T cells (13 - 16). This will be later investigated. The second assumption namely the exquisite radiosensitivity of a definite population of cell surface glycoconjugates need further work too. Preliminary experiments showed an increase in the protein, hexose and sialic acid contents in the irradiated-cells supernatants. Whether this released material represents a whole loosely associated membrane glycoconjugate or represents fragments of membrane closely associated glycoconjugates still remains to be determined.

Beside the release of sialic acid in the supernatants within oligosaccharides, glycopeptides or microvesicles, several other hypothesis can be envisionned to explain the decrease in membrane sialyl residues from irradiated cells. The translocation of sialic acid molecules from the peripheral to the deeper zones of the gly

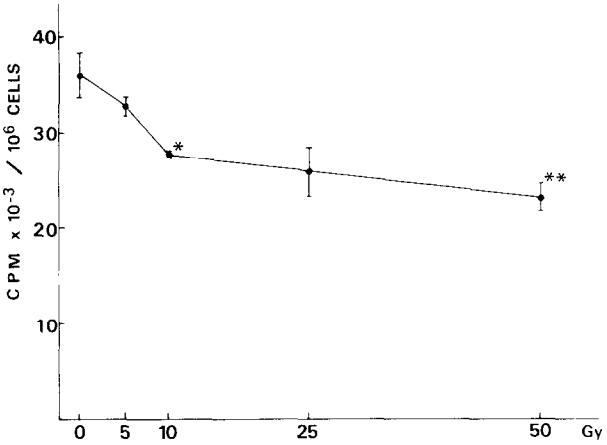

Figure 2 : Effect of increasing radiation doses (in grays : Gy) on the membrane periodate-sensitive sialic acid amount in mouse splenocytes. Sialic acid amounts are expressed as cpm per 10^6 cells.
Each point is the mean of two determinations. The bars represent two standard deviations. The data collected in the figure are from a single experiment which is representative of the overall study.
* $p < 0,05$ ** $p < 0,02$ (Student t-test)

cocalyx has been proposed (8). However, this seems unlikeley since we found that the periodate-borotritide procedure labels sialyl residues from gangliosides, acidic glycolipids deeply embedded in the glycocalyx (unpublished results).

We cannot exclude the possibility of a radiation-induced rearrangement of the sialic acid molecule expressed by a non reactivity towards periodate.

Our finding of a decrease in surface sialyl residues may be responsible for the post-irradiation decrease in the cells electronegativity as visualized by cell electrophoresis although no significant sialic acid release could have been demonstrated (5).

Our demonstration of the membrane partial desialylation in irradiated lympho cytes might explain their enhanced hepatotropism occuring immediately after the *in vitro* irradiation for B cells and after a short post-irradiation incubation for T cells (17).The liver entrapment of irradiated lymphocytes might then be tentatively compared with the neuraminidase-treated lymphocytes and asialoglycoproteins ones. In the latter cases the newly exposed galactosyl residues interact with the Hepatic Binding Protein from the hepatocyte membrane (18 - 20).

The finding of a very early radiation-induced membrane alteration may explain the drastically reduced blood mean residence time of irradiated lymphocytes (1).

It still remains to be demonstrated whether desialylation of peripheral blood lymphocytes and hepatic accumulation of these cells occur in mammals exposed to ionizing radiations.

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