ISOENZYME SPECTRUM OF LACTATE DEHYDROGENASE IN XENOGENIC RADIATION CHIMERAS

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It is shown that donors' cells can be identified in the body of rat – mouse radiation chimeras by investigating the lactate dehydrogenase isoenzyme spectrum.

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To study the life span and functional state of donors' hematopoietic cells in the recipient's body, methods of identification of chimerism must be improved.

The object of the present investigation was to study the possibility of using isoenzyme labeling to detect donors' cells in various organs of rat – mouse radiation chimeras [7-9]

EXPERIMENTAL METHOD

Adult C3H mice were irradiated on the RUM-11 apparatus with a total dose of 850 R (skin-focus distance 60 cm, filter 0.5 mm Cu + 1 mm Al, current 10 mA, voltage 180 kV, dose rate 7 R/min). A suspension of nucleated bone marrow ($2.8 \cdot 10^7$) and spleen ($5.2 \cdot 10^7$) cells from Wistar rats was injected intravenously into the irradiated mice 24 h later. The method of preparation of the cell suspension and of determining the viability of the cells was described previously [2]. Phosphatase labeling [3] was used to identify cells of the rat myeloid series in organs of the chimeras. Mice were sacrificed 7, 30, and 60 days after transplantation. Analysis of the lactate dehydrogenase (LDH) isoenzyme spectrum took place in several stages.

1. The organs were carefully washed to remove blood and minced in the cold in a glass homogenizer for 2 min at 2000 rpm in veronal – medinal buffer, pH 8.6, in the following proportions: heart 150 mg/ml, kidney 150 mg/ml, brain 350 mg/ml, liver 200 mg/ml, spleen 300 mg/ml. Bone marrow was obtained by repeated irrigation of the epiphyses of four femora with buffer solution in a volume of 0.25 ml. After washing, the erythrocytes were resuspended in buffer and hemolyzed by repeated freezing and thawing. The homogenates were centrifuged in the cold at 6000 rpm for 20 min and the supernatant was subjected to analysis.

2. Electrophoretic separation of the isoenzymes took place in agar gel [10] at 4° for 2.5 h at a voltage of 60 V and with a current of 50 mA; veronal – medinal buffer, pH 8.6, ionic strength 0.05. The test sample (0.02 ml) was placed in the groove at the starting line.

3. To determine the isoenzyme spectra, the agar blocks were incubated for 2 h in a substrate mixture of the following composition: 7 ml 0.5 M sodium lactate solution, 25 mg NAD, 12 mg/3 ml tetranitroblue tetrazolium, 5 mg phenazine metasulfate, 40 ml phosphate buffer, pH 7.4.

The ratio between the fractions was determined in percentages by densitometry. Total LDH activity was investigated by the method of Hill and Levy [6].

Irradiated mice protected by isologous bone marrow cells in a dose of $2.2 \cdot 10^7$–$2.5 \cdot 10^7$ were used as the control.

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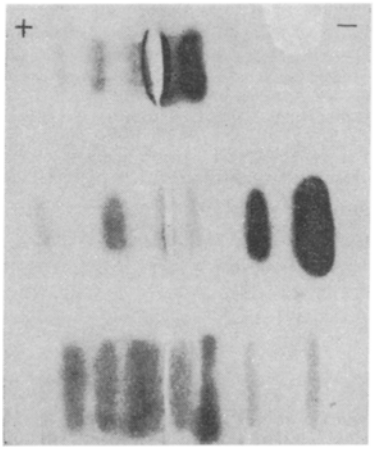

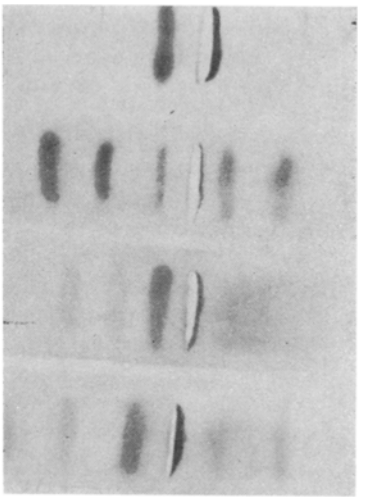

Fig. 1 Fig. 2

Fig. 1. Isoenzymes of LDH in bone marrow tissue. From top to bottom: enzymograms of control mouse, rat, and 30-day rat – mouse chimera. Explanation in text.

Fig. 2. LDH isoenzymes in spleen. From top to bottom: enzymograms of control mouse, rat, 30-day rat – mouse chimera, 60-day chimera. Explanation in text.

EXPERIMENTAL RESULTS

Transplantation of rat spleen cells into 14 lethally irradiated mice prolonged their life by only two weeks. Of the 38 irradiated mice protected by rat bone marrow cells, 18 died during the first week after transplantation. After 30 days there were only 12 viable chimeras, and after 60 days only 5 (13%). After injection of isologous bone marrow, 3 of the 12 mice died during the first week, but thereafter no other mice died.

The following considerations indicate that it is possible, in principle, to use isoenzyme labeling to identify donors' hematopoietic cells in xenogenic rat – mouse chimeras. First, definite differences were found in the relative percentages of individual fractions of LDH isoenzymes. This was particularly noticeable in the liver and spleen, where high LDH-5 activity was recorded in mice after fractionation in agar gel, wheras the other components of the spectrum were hardly formed at all. Under the same conditions of fractionation, 5 LDH isoenzymes are always found in the liver and spleen of rats. Second, LDH isoenzymes of rats and mice differ in their electrophoretic mobility. This applies above all to the cathode fractions LDH-4 and LDH-5. In mice they move only a short distance away from the starting zone, whereas in rats they are found nearer to the cathode. Since their locations on enzymograms do not coincide, when present in a single substrate they can be detected independently. Differences between the mobilities of the anode fractions LDH-1 and LDH-2 and the intermediate fraction LDH-3 were less marked. The locations of these fractions in the same enzymograms must partially overlap, so that their separate identification presents considerable difficulties.

Because of a decrease in total LDH activity in the organs and tissues of the 7-day radiochimeras, it was impossible to obtain definite LDH isoenzyme spectra. However, 30 days after transplantation of rat bone marrow cells, characteristic changes in the spectrum were observed in the bone marrow, spleen, liver, and circulating erythrocytes.

It is clear from Fig. 1 that a mixed LDH spectrum, consisting of 6 fractions, was present in the bone marrow of the chimeras at these times. Comparison of the enzymograms of chimera, intact rat, and mouse showed that the two extreme cathode fractions correspond in electrophoretic mobility to rat LDH-4 and LDH-5. The remaining components of the spectrum could either be formed by mouse isoenzymes or be

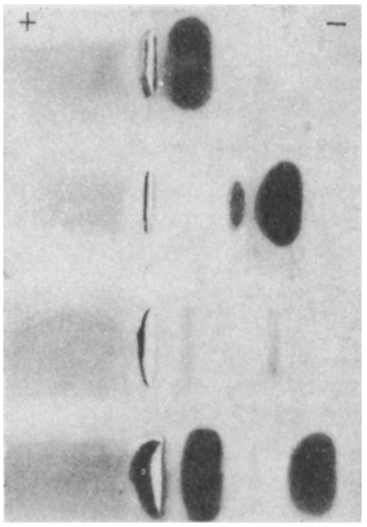

Fig. 3. LDH isoenzymes in liver. Order of enzymograms as in Fig. 2. Explanation in text.

hybridization products of isoenzymes of donor and recipient. In the 60-day chimeras the same spectrum of LDH isoenzymes on the whole was preserved in the bone marrow tissue.

It can be seen from Fig. 2 that besides mouse fraction LDH-5 (closer to the starting zone), isoenzymes LDH-4 and LDH-5 were formed in low concentrations in the spleen of the 30-day chimeras, and synthesis of anode fractions of donor origin had begun. In 60-day chimeras the new LDH isoenzyme spectrum was stabilized. The mouse LDH-5 fraction was still present, but four new stained zones had appeared, corresponding to the localizations of rat LDH-1, LDH-2, LDH-4, and LDH-5. Possibly rat LDH-3 coincided with mouse LDH-5. Summation of the spectra of donor and recipient thus took place in the spleen of the chimeras.

It is interesting to note that the gradual formation of isoenzymes of donor type also was observed in the liver tissue of the chimeras. Since total LDH activity in the liver was considerably reduced after irradiation, the two faintly stained zones were detected with great difficulty on the enzymograms of the 30-day chimeras. Nevertheless, it was seen that, besides mouse LDH-5, a fraction which, by its localization, belonged to rat LDH-5, appeared (closer to the cathode). The components of the spectrum donor and recipient were clearly differentiated 60 days after transplantation (Fig. 3).

Two LDH fractions were found in the erythrocytes of the chimeras, one of which was connected with mouse erythrocytes and located closer to the starting zone, while the second was formed by rat erythrocytes. In the other tissues investigated (heart, kidney, brain) and in the blood serum, no "chimeric" spectra of LDH isoenzymes could be detected.*

Identification of the cells which were responsible for forming the donors' lines in the LDH isoenzyme spectrum of the bone marrow, spleen, and liver of the rat – mouse radiochimeras was not among the objects of this investigation. Nevertheless, the results indicate that the study of the LDH isoenzyme spectrum can be a convenient method for investigating the distribution and periods of persistence of donors' hematopoietic cells in the body of radiochemieras. One advantage of this method of identification of chimerism is its high resolving power and its universal character because it can be used successfully to detect cells of the erythrocyte, myeloid, and lymphoid series. By means of an isoenzyme marker, the relative proportions of cells of these series belonging to donor and recipient can be estimated with a high degree of accuracy at different times after transplantation.

The use of an isoenzyme marker, combined with other methods of identification of donors' cells using phosphates [1, 3], immunologic [5], and genetic [4] markers thus provides a means of obtaining a more complete picture of types of chimerism under concrete experimental conditions.

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*Because of atrophy of the thymus and lymph glands, isoenzymes were not studied in these organs.