UTILIZATION OF Cr^{51}-LABELED LYMPHOCYTES IN VIVO

Yu. I. Zimin and A. F. Filinkov

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The kinetics of lymphocytes from the thymus and lymph glands, labeled in vitro with Cr^{51}, was investigated in experiments on mice. A radiometric investigation of the whole body showed that the loss of specific activity of the viable cells was the sum of two components, the first with a half-period of three days for the thymus and five days for the lymph glands; the second component was approximately the same for these cells, namely 41 days. The kinetics of the labeled nonviable cells was found to have a similar structure and to differ only in its first component. Through the reutilization of Cr^{51} it was therefore impossible to draw any conclusions regarding the distribution and the life span of the thymus and lymph gland cells.

Investigation of the behavior of labeled lymphocytes in vivo is directly linked with the solution of a complex technical problem: how to avoid possible error through reutilization and recirculation of the cells.

The method of tagging lymphocytes with Cr^{51} developed in recent years has many advantages. First, at the moment of incorporation of sodium chromate ($Na_2Cr^{51}O_4$) the hexavalent chromium ion is converted into trivalent and, as experiments [2, 4] have shown, in this form it is not absorbed by other cells; second, Cr^{51} emits γ rays, so that the labeled cells are accessible for external indication where they were found. However, conclusions regarding the inability of Cr^{51} to be reutilized are based entirely on investigations of circulating blood cells [2, 4]. This was the reason why Cr^{51} has been recommended as the most promising marker in hematological research.

The kinetics of transplanted viable and nonviable thymus and lymph gland cells, labeled in vitro with Cr^{51}, was investigated. Reutilization of Cr^{51} was shown to take place in the living organism.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL) F_1 mice from the Stolbovaya Nursery of the Academy of Medical Sciences of the USSR. The mice were decapitated. Donor suspensions of thymus and lymph gland cells were obtained as described earlier [1]. Their content of erythrocytes did not exceed 1%. The lymphocytes were marked with Cr^{51} [4, 5]. The cells were incubated in Hanks' medium with sodium chromate $Na_2Cr^{51}O_4$ solution (specific activity 2.4-4.2 mCi/ml, pH 7.4) for 30 min at 37° with constant stirring. The concentration of sodium chromate during incubation did not exceed 1 μg/ml. After incubation the cells were washed twice in medium No. 199. The ascorbic acid contained in this medium converted the free hexavalent chromium into trivalent, thereby stopping the absorption of chromium by the cells.

Viable (not staining in 0.1% trypan blue solution) labeled lymphocytes were transplanted intravenously into mice of the same line in a dose of 7×10^7 to 8×10^7 cells.

The same number of labeled cells was heated to 60° C for 25 min before transplantation. Special experiments showed that after heating the cells of the thymus and lymph glands completely lost their viability.

The corresponding number of labeled cells was applied to a target and served as the reference during subsequent measurements for the experimental radiometric assays.

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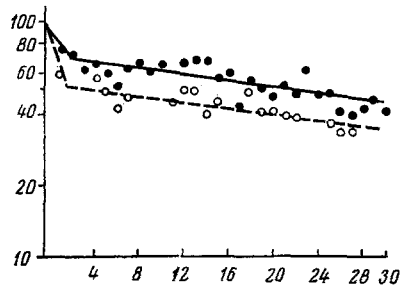

Fig. 1

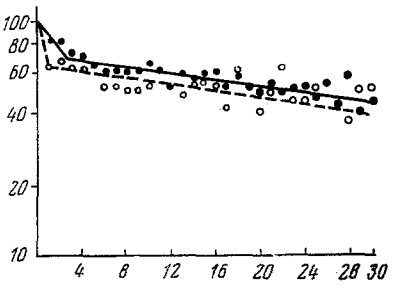

Fig. 2

Fig. 1. Radioactivity of the whole body after transplantation of viable (continuous line) and nonviable (broken line) thymus cells labeled in vitro. Abscissa, time, (in days) after transplantation; ordinate, pulses/min (in % of reference and of original value).

Fig. 2. Radioactivity of whole body after transplantation of viable (continuous line) and nonviable (broken line) lymph gland cells labeled in vitro with Cr^{51}. Legend as in Fig. 1.

The values obtained relative to the reference in percentages of the initial level are shown in Figs. 1 and 2.

Immediately after injection of the cells and daily thereafter a radiometric investigation of the whole body and of the reference was carried out on the Gamma (Hungary) apparatus, consisting of ND-108 counter, NK-131 scintillation measuring head, with NaI crystal and NZ-136 collimator. During the investigation the amplitude discriminator of the counter worked on a differential program, corresponding to the energy of the γ-ray emission of the isotope Cr^{51}. The geometry of counting was constant during the measurements. For this purpose each mouse was placed in a glass container with a capacity of 40 cm^3.

The experiments were repeated three times; each point on the graph in Figs. 1 and 2 is the mean value for 10 animals.

EXPERIMENTAL RESULTS

The results of investigation of the radioactivity of transplanted Cr^{51}-labeled thymus and lymph gland cells showed (Figs. 1 and 2) that the decrease in radioactivity of the viable cells was the sum of two components: the first with a half-period of three days for the thymus and five days for the lymph glands, the second with a half-period approximately equal for the thymus and lymph glands (41 days).

The experiments showed that the kinetics of the labeled nonviable cells was similar in its pattern, differing only in the first component. For instance, the first phase had a half-period of 1.5 days regardless of the nature of the cells, while the second component, as for the viable cells, was 41 days. The small differences in the first phase compared with the viable cells must evidently be regarded as the result of increased elution of the trivalent chromium as the result of heat treatment of the lymphocytes [3].

Samples of whole blood of mice taken at the end of the investigation contained no labeled cells.

Little is yet known about how the Cr^{6+} ion binds with the cells. All that is known is that hexavalent chromium, on binding with the cells, becomes trivalent, and the free trivalent ion is not utilized by other cells [2, 4, 5]. The experimental results suggest that after death of the labeled cells the chromium ion does not become free but is incorporated into metabolism in the bound form. Consequently, the labeled transplanted lymphocytes survive to maturity, disintegrate, and their fragments are absorbed by other tissues. The most probable explanation is that the dying lymphocytes are phagocytosed by elements of the reticulo-endothelial system.

As the result of utilization of Cr^{51}-labeled lymphocytes it was thus impossible by the use of this method to draw any conclusions regarding the life span or distribution of the thymus and lymph gland cells. It can be confidently stated that circulating blood cells and their precursors do not utilize "fragments" of Cr^{51}-labeled lymphocytes. This is shown by the results of investigations of blood samples.

The results of these experiments show that radiometric measurements from the body surface cannot be used to study the kinetics of Cr^{51}-labeled lymphocytes. This method can be used only to study circulating lymphocytes.

LITERATURE CITED

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