# USE OF $Cr^{51}$-LABELED PERITONEAL MACROPHAGES GROWN *in vitro* AS TARGET CELLS FOR QUANTITATIVE ANALYSIS OF CYTOTOXIC ACTIVITY OF IMMUNE T-LYMPHOCYTES

G. I. Drizlikh, A. V. Andreev,
I. F. Kotomina, and B. D. Brondz

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The use of macrophages previously labeled with $Cr^{51}$ in suspension and growing in a monolayer on coverslips treated with poly-L-lysine (PLL), as target cells provides a means of determining the cytotoxic effect (CE) of immune lymphocytes based on the liberation of $Cr^{51}$, which in the control does not exceed 10-20% of the maximal. The method proved also to be suitable for determining the effectiveness of lymphocyte fractionation. By the use of 2% sodium dodecylsulfate solution, 100% solubilization of labeled macrophages growing on the surface of the coverslip could be achieved, and CE could also be determined by measuring the quantity of label left behind in the intact macrophages. Both methods were found to be accurate, reproducible, and sensitive and they correlated closely both with each other and with the method of direct cell counting.
KEY WORDS: *cytotoxic effect; T-lymphocytes; macrophages; poly-L-lysine; sodium dodecylsulfate.*

TABLE 1. Effectiveness of $Cr^{51}$-Labeling of Macrophages from Different Strains of Mice and Liberation of $Cr^{51}$ during Incubation with Normal Lymphocytes (M±m)

| Strain of mice | Total radiation (counts/min) | NL (in % of TR) |
|---|---|---|
| B10 | 3125±261 | 15,1±0,3 |
| C57BL/6 | 3209±196 | 16,4±1,7 |
| A | 2526±250 | 18,7±1,6 |
| DBA/1 | 3158±79 | 13,3±1,8 |
| R103 | 4244±39 | 14,5±1,7 |
| Hz1 | 3838±73 | 10,2±0,2 |
| M505 | 3548±17 | 14,8±0,3 |

Legend. $4 \times 10^5$ MP-$Cr^{51}$ were incubated for 48 h, washed 3 times with 199 LB, after which TR was determined in some samples and NL in others after incubation for 20 h at 37°C with $8 \times 10^6$ normal allogeneic lymphocytes.

During the study of the specificty of transplantation immunity the need arises for estimating the cytotoxic effect (CE) of immune lymphocytes *in vitro* relative to target cells from a wide variety of mouse strains. The use of $Cr^{51}$-labeled tumor cells as target cells [5] is unsuitable for this purpose because of great differences in the sensitivity of different tumors to CE.

In the investigation described below the possibility of cultivating peritoneal macrophages labeled with $Cr^{51}$ was studied, with the object of replacing direct counting of target cells left on the coverslip after incubation with lymphocytes [1-3] by measuring their radioactivity or determining their CE on the basis of liberation of $Cr^{51}$ into the medium.

## EXPERIMENTAL METHOD

Mice of several inbred strains obtained from the nursery of the N. F. Gamaleya were used. Sarcomas Sal and MChll, induced by carcinogens respectively

Laboratory of Chemistry and Synthesis of Antibodies and Laboratory of Immunochemistry and Diagnosis of Tumors, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. V. Vygodchikov.) Translated from Byullenten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 3, pp. 340-342, March, 1976. Original article submitted March 24, 1975.

TABLE 2. Comparison of Three Methods of Determination of Cytotoxic Effect of Immune Lymphocytes Using $Cr^{51}$-Macrophages as Target Cells*

| Dose of immune lymphocytes | Cytotoxic effect (%)† | | |
|---|---|---|---|
| | liberation of $Cr^{51}$ | retention of $Cr^{51}$ | counting cells |
| $2\times10^6$ | 23,3 | 35,5 | 35,4 |
| $4\times10^6$ | 62,1 | 61,3 | 70,6 |
| $8\times10^6$ | 88,3 | 88,6 | 87,3 |

*Immune lymphocytes obtained from $B10.D_2$ mice immunized with sarcoma MChll, normal lymphocytes obtained from normal $B10.D_2$ mice, macrophages from B10 mice.
†$P < 0.001$ in all cases

in mice of strains A and C57BL/10 (abbreviated to B10), were maintained in the ascites form by weekly passages and were used to immunize B10 and $B10.D_2$ mice respectively at five subcutaneous points and also intraperitoneally ($40 \times 10^6$-$50 \times 10^6$ cells per mouse). The methods of obtaining and cultivating the peritoneal macrophages, of preparing the lymphocyte suspension, and of carrying out the CE experiments were fully described previously [1-3]. Macrophages ($10 \times 10^6$ ml) were labeled with $Cr^{51}$ as described in [5] in medium No. 199 containing 10% calf embryonic serum (CES), heparin (25 units/ml), 0.005 M Hepes, and $Cr^{51}$ (50-75 μ Ci) in an atmosphere of 5% $CO_2$ for 1 h with periodic mixing. The labeled macrophages (MP-$Cr^{51}$), washed to remove unbound label, were suspended at the rate of $4 \times 10^5$ cells/ml in medium No. 199 with 10% bovine serum, 10% lactalbumin (199 LB), and 0.005 M Hepes, and added in a volume of 1 ml to Leighton's tubes, previously treated with poly-L-lysine (PLL) [12]. After incubation for 48 h at 37°C in Leighton's tubes treated with PLL, the MP-$Cr^{51}$ were washed 3 times with 199 LB, with vigorous shaking. To determine total radioactivity of the MP-$Cr^{51}$ in some of the samples, 1 ml of a 2% solution of sodium dodecylsulfate in 0.05 M borate, containing 0.002 M EDTA was added to the cultures and, after incubation for 30 min, the contents of the samples were transferred quantitatively to tubes for measurement of radioactivity in the Nuclear Chicago Gamma-Spectrometer. Treatment with sodium dodecylsulfate led to 100% solubilization of the MP-$Cr^{51}$. The remaining samples were treated with $2 \times 10^6$ to $8 \times 10^6$ normal or immune lymphocytes suspended in 1 ml of medium No. 199 with 5% CES and 0.05 M Hepes and incubated for 20 h at 37°C, after which the CE of the immune lymphocytes was estimated as follows: a) by direct counting of the cells under the microscope, using the formula

$$\frac{a-b}{a}\times 100,$$

where a and b are the mean number of living MP-$Cr^{51}$ remaining on the coverslip after incubation with normal and immune lymphocytes respectively; b) by measurement of the retention of $Cr^{51}$ by the formula

$$\frac{NR - IR}{NR} \times 100,$$

where NR and IR represent the radioactivity of MP-$Cr^{51}$ remaining on the coverslip after incubation with normal and immune lymphocytes respectively, washed off, and solubilized with sodium dodecylsulfate; c) by measuring the liberation of $Cr^{51}$ by the formula

$$\frac{(IL - NL)}{(TR - NL)} \times 100,$$

where NL and IL represent the quantity of $Cr^{51}$ respectively in the culture medium mixed with two portions, each consisting of 0.5 ml 199 LB used to wash each culture, after incubation of the MP-$Cr^{51}$ with normal and immune lymphocytes respectively; TR denotes total radioactivity. The significance of differences was assessed by Student's t-test.

## EXPERIMENTAL RESULTS

Under the conditions of labeling and cultivation of MP-$Cr^{51}$ as described, NL did not exceed 10-20% of the total radioactivity after incubation for 20 h, regardless of the strain of mice used as the source of macrophages (Table 1). The result of one experiment in which the three methods of determining CE were compared is shown in Table 2. Clearly the values of CE determined by direct counting of the residual target cells agreed well with those obtained by measurement of their radioactivity and of the radioactivity liberated from them.

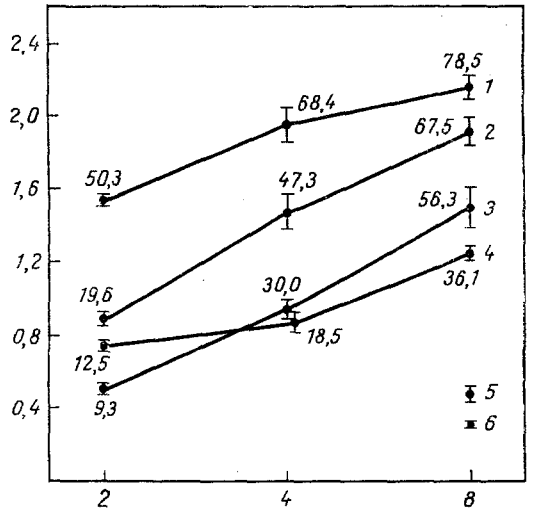


Fig. 1. Cytotoxic effect of B10 anti-A lymphocytes and their T- and B-fractions after incubation for different times with MP-$Cr^{51}$ from strain A. Abscissa, dose of immune lymphocytes ($\times 10^6$); ordinate, liberation of $Cr^{51}$ (in counts/min) ($\times 10^3$). Numbers by points on curves show CE (in %). Vertical lines give standard error ($\times$ 2). Maximal liberation of $Cr^{51}$ was 2413 counts/min. 1) Immune lymphocytes, T-fraction, incubation for 20 h; 2) immune lymphocytes, unfractionated, incubation for 20 h; 3) immune lymphocytes, unfractionated, incubation for 4 h; 4) immune lymphocytes, B-fraction, incubation for 20 h; 5) normal lymphocytes ($8 \times 10^6$), incubation for 20 h; 6) normal lymphocytes ($8 \times 10^6$), incubation for 4 h.

A 50% level of CE, determined from the liberation of $Cr^{51}$, was found after incubation of immune lymphocytes with MP-$Cr^{51}$ for 4 h (Fig. 1). As Fig. 1 also shows, the dose of immune lymphocytes required for development of 50% CE was halved if the lymphocyte population was enriched with T-cells as a result of fractionation on a monolayer of sheep's red cells, coated with antierythrocytic antibodies [9]. On the other hand, the cytotoxic activity of B-fraction was considerably reduced.

The method described above has advantages over the use of lymphocytes stimulated by PHA and labeled with $Cr^{51}$ as target cells, which have the disadvantage that their NL value is high, amounting to 50% of the maximal liberation [8, 11]. The same result was observed in the present investigation if macrophages were labeled with $Cr^{51}$ in each individual 3-day culture [7] or even immediately after their removal from the peritoneal cavity, but if they were used as target cells immediately after labeling [6]. Other normal cells labeled with $Cr^{51}$, either fibroblasts or thymocytes, are less sensitive to the CE of lymphocytes than macrophages [10].

The method described above is thus sufficiently accurate, sensitive, and reproducible and it is suitable for determining CE on target cells of different lines of mice, as well as the effectiveness of fractionation of the lymphocytes. The results obtained with its aid during a study of the specificity of receptors of T-lymphocytes correlated closely with the results obtained by the method of direct cell counting [4].

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