FREQUENCY OF SISTER CHROMATID EXCHANGES IN CULTURED MOUSE BLOOD, BONE MARROW, AND SPLEEN CELLS

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KEY WORDS: sister chromatid exchanges; mouse, spleen; blood; bone marrow.

The method of counting sister chromatid exchanges (SCE) is highly sensitive, and it can be used to determine the action of factors present in low intensity. The number of SCE in bone marrow cells of, for example, the rabbit has been shown to be about three to four exchanges per cell [4]. Meanwhile the spontaneous level of SCE in rabbit peripheral blood lymphocytes cultured in vitro is seven to 10 exchanges per cell [1]. It is not yet clear what causes this great difference in the number of SCE when counted in vivo and in vitro. It may perhaps be due to the fact that SCE are counted in different tissues. It can also be suggested that the number of SCE is influenced by the concentration of brominated DNA precursors and also by the duration of their presence during cell culture.

To estimate the effect of these factors on the SCE level it was decided to study their number in cultured cells of different origin (from the spleen, bone marrow, and blood), and also on the concentration of 5-bromodeoxyuridine (BDU) and 5-bromodeoxycytidine (BDC) and on the duration of culture.

EXPERIMENTAL METHOD

For each variant of the experiments two or three male BALB/c mice were used. After sacrifice of the animals blood was aspirated from the pericardial sac by means of a syringe wetted in heparin. Bone marrow cells were flushed out of the femora with 5 ml of Hanks' solution (37°C). The animals' spleens were homogenized in 3 ml of medium 199, the homogenate was allowed to stand for 5 min to sediment pieces of tissue, the cell suspension was transferred to a centrifuge tube, the volume made up to 10 ml, and the sample centrifuged for 10 min at 400 g. The supernatant was poured off and the cells were suspended. From two or three mice altogether 5×10^6 cells from both spleen and bone marrow of each were transferred into 5 ml of medium of the following composition: 90% medium RPMI-1460, 10% embryonic calf serum, 2×10^{-3} M HEPES, 2.8×10^{-4} M 2-mercaptoethanol, 50 μg/ml gentamicin, 40 μg/ml concanavalin A, and 10 μg/ml of BDU or BDC. To 0.5 ml of whole blood 4.5 ml of medium of the same composition was added. The bone marrow cells were cultured without concanavalin A. The cells were cultivated in a lightproof container at 37°C in an atmosphere containing 5% CO_2. Colcemid (0.1 μg/ml) was added 1 h before fixation. Preparation and staining of the specimens were described previously [2]. In each variant 25 metaphases were analyzed.

EXPERIMENTAL RESULTS

Table 1 gives the number of SCE in the spleen cells during culture with different concentrations of BDU and BDC for 48 h. It was shown by two-factor dispersion analysis that the number of SCE per cell does not depend on the type of brominated DNA precursor or on their concentration ($p > 0.05$ for both factors), and averages 10.27 exchanges per cell. This is much higher than the frequency of SCE discovered in vivo in mouse bone marrow (3-4 exchanges per cell [4]). Nevertheless, the experimental results demonstrate that lowering the BDU or BDC concentration form 10 to 1 μg/ml has no effect whatever on the number of SCE during culture in vitro. It can accordingly be postulated that the increased number of SCE during culture compared with their number in vivo has nothing to do with the BDU concentration.

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TABLE 1. Mean Number of SCE per Cell (± standard error) in Cultured Spleen Cells Treated with Different Concentrations of BDU and BDC

Concentration, μg/ml	Brominated DNA precursor	
	BDU	BDC
1	11,08±0,78	10,04±0,67
3	10,24±0,87	11,88±1,06
10	12,32±1,01	9,68±0,53

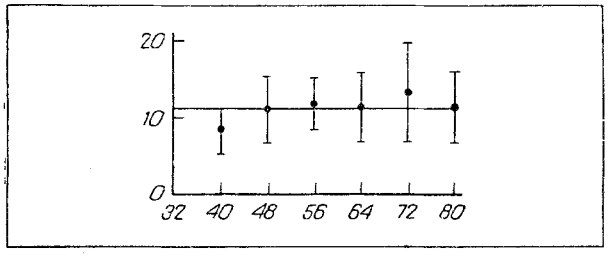

Fig. 2. Frequency of sister chromatid exchanges in spleen cells during culture for different times. Abscissa, duration of culture, h; ordinate, number of SCE per cell. Mean values with 95% confidence intervals and overall average level are shown.

TABLE 2. Number of SCE in Cells from Different Tissues in Culture

Tissue	Number of SCE per cell ± standard error
Blood	
mouse I	10,44±1,06
mouse II	10,44±1,01
Bone marrow	12,68±1,10
Spleen	11,00±0,84

Another possible factor affecting the number of SCE may be the duration of culture. Its role was tested in an experiment in which spleen cells were fixed from the 40th until the 80th hour of culture. The results are given in Fig. 1 and show that the mean number of SCE per cell for each experimental point corresponded, within 95% confidence limits, to the average level (11.18 SCE per cell). No correlation was found between the number of SCE and the duration of culture; regression was not significant ($F = 4.78$, $df = 4$, $p > 0.05$). The components of the culture medium thus do not give rise to an increase in the number of SCE. Otherwise, with an increase in the duration of exposure to them, the number of SCE also would increase.

Comparison of the frequency of SCE in spleen, blood, and bone marrow cells cultured for 48 h gave results shown in Table 2. Unlike bone marrow and spleen cells, blood lymphocytes from different animals were cultured separately. Analysis by Student's test showed no difference in the frequency of SCE for all three tissues ($p > 0.05$). Consequently, the frequency of SCE in cells from actively proliferating tissues (bone marrow) was the same as in tissues in which the cells were basically in the resting state, but began to divide in vitro under the influence of mitogenic agents.

The results are evidence that neither the origin of the tissue nor the duration of culture with different concentrations of BDU or BDC, nor the use of mitogens to stimulate division

in vitro are factors which lead to an increase in SCE in cells in culture. It can be postulated that it is the actual procedures of isolation of the cells and obtaining of a primary culture which lead to an increase in the number of SCE in cells in culture compared with the number estimated in vivo. Evidence in support of this conclusion is given by the fact that the frequency of SCE in lymphocytes during culture of whole blood averages 8.5 per cell [3], but when the additional procedure of isolating the cells with Ficoll is used the number of SCE increases to 11.4 exchanges per cell [5].

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SYNERGIC ACTION OF LIPOPOLYSACCHARIDE AND MURAMYL DIPEPTIDE IN IMMUNOTHERAPY OF DBA/2 MICE WITH MASTOCYTOMA P815

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-092.9-085.275.4.015.21

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KEY WORDS: lipopolysaccharide; muramyl dipeptide; immunotherapy.

One of the most promising and effective methods of tumor immunotherapy is considered to be the use of combinations of immunomodulators with different mechanisms of action on cells of the immune system, and for that reason capable of potentiating each other's effect [8]. The writers previously demonstrated the synergic action of immunomodulators of bacterial origin, namely lipopolysaccharide (LPS) and muramyl dipeptide (MDP), on activation of the production of tumor necrosis factors (TNF) and of interleukin-1 in mice in vitro [4]. It was shown that the combined use of LPS and MDP can induce necrosis and regression of subcutaneous nodes of highly immunogenic syngeneic tumors: sarcoma Meth A in BALB/c mice [6] and sarcoma SA-1 in A/Sn mice [5]. This paper describes a study of the effect of a combination of LPS and MDP on growth of syngeneic mastocytoma P815, which has low immunogenicity, in DBA/2 mice. The cyclo-oxygenase inhibitor indomethacin and the cytostatic cyclophosphamide also were used for immunotherapy.

EXPERIMENTAL METHOD

Male DBA/2 (H-2^d) mice weighing 18-20 g were used. Injection of 10^6 mastocytoma P815 (H-2^d) cells, maintained in vivo by weekly passages in DBA/2 mice, was given subcutaneously into the right side. After 5 days, either LPS of E. coli 055:B5 ("Difco," USA) in a dose of 5 μg per mouse or MDP (obtained from N. V. Bovin, Institute of Bioorganic Chemistry) in a dose of 15 μg per mouse, or a combination of LPS and MDP, in 0.2 ml of medium 199 was injected at the site of injection of the tumor cells; some mice were injected with tumor cells only. Immunotherapy was given 5 times at intervals of 2-3 days (experiments of series I). In series II the mice were divided into seven groups with 10 animals in each group. A mixture

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