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# ACTION OF ALLOGENEIC AND SYNGENEIC SPLENIC EXTRACTS ON PRIMARY CELL CULTURES FROM INBRED MICE

I. P. Kosova and I. I. Podoplelov

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The effect of allogeneic and syngeneic extracts from the spleens of male and female inbred mice on primary cultures of fibroblasts obtained from the subcutaneous connective tissues of fetuses of CBA and C57BL/6J mice was studied. The cytotoxic and growth-inhibiting action on the cultures was successively enhanced by the use of extracts from syngeneic male and allogeneic female and male tissues. Consequently, an increase in the degree of antigenic difference between the target cells and extracts led to enhancement of the phenomenon of allogeneic inhibition. It was shown for the first time that in a syngeneic system extracts from male tissues (containing the weak H–Y antigen) have a cytotoxic action on cells from female inbred mice, i.e., that they induce a reaction of the allogeneic inhibition type.

KEY WORDS: target cell; extract; antigen; allogeneic inhibition.

Extracts that are foreign with respect to strong tissue compatibility antigens are known to have a cytotoxic action (allogeneic inhibition) on target cells in culture [2, 4, 5, 8, 9]. However, no data could be found in the accessible literature on the effect of extracts on target cells differing in the weak H–Y antigen. The problem of whether in this case the reaction is of the allogeneic inhibition type and whether this effect increases with an increase in the antigeneic differences between the target cells and extracts has not yet been studied.

The object of this investigation was to compare the cytotoxin effect of extracts differing with respect to strong (H–2) and weak (H–Y) transplantation antigens on primary cultures of cells from inbred mice.

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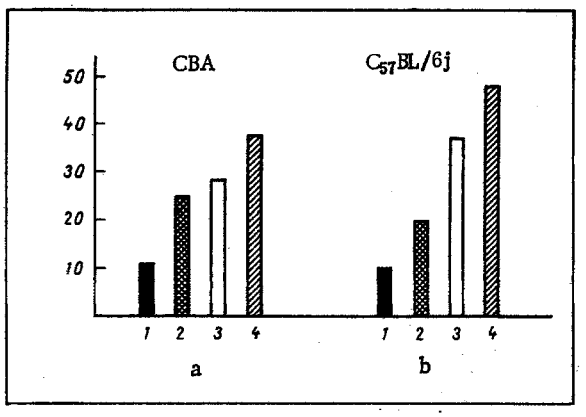


Fig. 1. Viability of primary cell cultures treated with different splenic extracts. Abscissa, action of different extracts; ordinate, number of dead cells (in %). Extracts from CBA (a) and C57BL/6J (b) cells respectively. 1) Syngeneic females, 2) syngeneic males, 3) allogeneic females, 4) allogeneic males. Differences between action of all extracts in experiment and control are statistically significant.

## EXPERIMENTAL METHOD

Primary cultures of fibroblasts obtained by the trypsinization method from the subcutaneous connective tissue of CBA and C57BL/6J mouse fetuses were used as the test system.

Extracts were prepared from spleens of adult female and male CBA and C57BL/6J mice. The spleens were removed under aseptic conditions, cut up finely with scissors, and ground in a glass homogenizer in Hanks' solution. The resulting cell suspension was frozen (in acetone with dry ice) and thawed several times, and then centrifuged at 1500g for 30 min. The supernatant, in which the protein concentration was determined by the Lowry's method [1], was used in the experiments. Primary cultures were grown at the rate of 200,000 fibroblasts in Wassermann tubes with 2 ml medium 199 and 1% syngeneic serum. After 24 h the nutrient medium was poured from the tubes and replaced by extracts in a dose of 50 μg of protein in 2 ml medium 199. The cells with the extracts were then incubated at 37°C for 48 h, after which the number of living and dead fibroblasts was counted in each tube, after staining with a 1% solution of the trypan blue. The coefficient of proliferation (CP) — the ratio between the number of cells grown and the number of cells seeded initially, and the percentage of dead target cells (the cytotoxic action — CTA) were calculated as criteria of growth and viability of the cell culture.

The numerical results were subjected to statistical analysis by the Fisher—Student method in Moshkovskii's modification [6]. Results were significant for which $P < 0.05$. The results of five series of experiments are given in Fig. 1 and Table 1.

## EXPERIMENTAL RESULTS

The mean indices of growth and viability of primary fibroblasts from CBA and C57BL/6J mice obtained under the influence of extracts differing with respect to strong and weak transplantation antigens are illustrated in Fig. 1 and Table 1. Primary cultures incubated with syngeneic mouse spleen tissue extract served as the control. The ♀CBA control culture contained 10.4 ± 0.43% of dead target cells, corresponding to a CP of 1.60 ± 0.03; the ♀C57BL/6J culture contained 9.7 ± 0.73% of dead fibroblasts, corresponding to a CP of 1.57 ± 0.05. The action of male syngeneic splenic extract led to an increase in the number of dead ♀C57BL/6J cells to 19.8 ± 0.85% ($P = 0.002$) and of ♀CBA cells to 24.8 ± 0.70% ($P = 0.001$). In the first case CP was 1.35 ± 0.02, and in the second 1.39 ± 0.02, i.e., it was statistically significantly lower than in the control ($P < 0.050$).

Consequently syngeneic extracts differing with respect to the weak H—Y antigen have a cytotoxic and growth-inhibiting action on target cells in culture.

Allogeneic extracts (differing from the target cells with respect to H—2 antigens) had a marked cytotoxic action. In this case the number of dead cells in ♀CBA cultures on incubation

TABLE 1. CP of Primary Cultures from CBA and C57BL/6J Mice Treated with Splenic Extracts from Male and Female Allogeneic and Syngeneic Mice

| Target cells of female mice | Extracts from undermentioned mouse spleens | CP | P |
|---|---|---|---|
| CBA | ♀CBA (syngeneic) | 1,60±0,03 | — |
| | ♂CBA (syngeneic) | 1,39±0,02 | 0,013 |
| | ♀C57BL/6 Y (allogeneic) | 1,31±0,05 | 0,003 |
| | ♂C57BL/6 Y (allogeneic) | 1,18±0,02 | <0,001 |
| C57BL/6J | ♀C57BL/6J (syngeneic) | 1,57±0,05 | — |
| | ♂C57BL/6J (syngeneic) | 1,35±0,02 | 0,010 |
| | ♀CBA (allogeneic) | 1,28±0,03 | 0,002 |
| | ♂CBA (allogeneic) | 1,11±0,04 | <0,001 |

with the ♀C57BL/6J splenic extract was 28.5 ± 0.71%, whereas on incubation of ♀C57BL/6J fibroblasts with splenic extract ♀CBA mice it was 37.3 ± 0.41%. CP was lowered to 1.31 ± 0.05 and 1.28 ± 0.03 respectively, and differed significantly from CP in the control cultures.

Allogeneic extracts from tissues of male mice differing from the target cells with respect to both H-2 and H-Y antigens simultaneously had the strongest cytotoxic and growth-inhibiting action on female cells. For instance, ♂C57BL/6J extract increased the number of dead CBA cells to 37.7 ± 0.09% and lowered CP of the culture to 1.19 ± 0.02. The ♂CBA extract was even more toxic for ♀C57BL/6J cells, for there were 48.7 ± 0.81% of dead fibroblasts and CP was 1.11 ± 0.04. Differences between the experimental and control indices in all cases with statistically significant ($p < 0.05$). Consequently, when target cells were treated with extracts differing from them with respect to the H-Y antigen and antigens of the H-2 locus or both these antigens together, the cytotoxic action increased regularly and was maximal in the third experiment, i.e., the phenomenon of allogeneic inhibition was enhanced by an increase in the degree of the antigeneic differences between the interacting systems. A similar effect has also been observed in the blast-transformation reaction [7].

The fact thus established, that target cells give a reaction of allogeneic inhibition type under the influence of extracts foreign with respect to the weak H-Y antigen is in agreement with Petrov's observations [3] that allogeneic inhibition is manifested when the interacting cells differ not only in strong tissue compatibility antigens.

In the present experiments the phenomenon of allogeneic inhibition appeared when target cells were treated under similar conditions by extract differing with respect to H-2 and H-Y antigens. These results may be useful for the development of new experimental models *in vitro* for the further study of natural and acquired immunity.

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