# EFFECT OF ANTISPLENIC SERUM ON STEM CELLS OF HEMATOPOIETIC TISSUE

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Mouse spleen cells were treated in vitro with rabbit antisplenic serum. The number of foci formed in the spleen of recipient mice after transplantation of treated cells into the animals was smaller than in the control (animals receiving spleen cells not treated with antiserum).

A vital problem in modern immunology is the control of the immunologic reactivity of the body. A factor of particular importance is the use of immunodepressants, especially antilymphocytic sera (ALS). These are widely used for the investigation of homografting [4, 5, 8, 13], antibody formation [2, 3, 11, 12], and the formation of delayed hypersensitivity [10, 14]. The effect of ALS is accompanied by its cytotoxic action on lymphoid cells. The effect of ALS on cells of different types, especially on stem cells of hematopoietic tissue, has received little study. The only findings which are known are concerned with the action of ALS on bone marrow [6].

The object of this investigation was to study the effect of antisplenic serum (ASS) on spleen cells in vitro. The ability of spleen cells to form clones in the recipient's spleen after transplantation into irradiated mice was investigated.

## EXPERIMENTAL

Inbred mice of lines CBA, CC57BL, and (CBA × C57BL)$F_1$, weighing 16-18 g, were used. The animals were irradiated with $\gamma$ rays in a dose of 830 rad ($LD_{100/12}$). ASS were obtained by immunizing rabbits with two intravenous injections of a suspension of mouse spleen cells by the method of Levey and Medawar [9]. The sera, inactivated by heat (30 min at 56°), were stored at −20°. The cytotoxic titer of the sera [1] was 1:256. In some cases the ASS was exhausted by erythrocytes and by erythrocytes and mouse kidney cells. Under these circumstances the cytotoxic titer was 1:32 and 1:16 respectively.

To obtain McCulloch's foci, $2.5 \times 10^6$ donor's spleen cells were transplanted intravenously into mice 4 h after irradiation. The transplanted cells were treated in vitro either by various dilutions of ASS (30 min at 37°) or by normal rabbit serum (NRS), or by Hanks's solution. Initially, before treatment with ASS, the suspension contained $20 \times 10^6$ cells/ml (about 90% of them viable). Before transplantation, the cells were washed by repeated centrifugation in Hanks's solution to remove ASS. After treatment with ASS the percentage of viable cells in the suspension was unchanged.

The animals were sacrificed 9 days later and the spleen removed and fixed in alcohol-formol. Macroscopic foci were counted in the fixed preparation.

## EXPERIMENTAL RESULTS

After intravenous transplantation of $2.5 \times 10^6$ spleen cells incubated in Hanks's solution, the mean number of foci formed in the spleen of the irradiated recipient was 10-17 (Table 1). The number of foci depended on the combination of donor and recipient, and indicated the exhibition of syngeneic preference. Treatment of

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TABLE 1. Formation of Foci in Spleen of Irradiated (830 rad) Recipient after Transplantation of Spleen Cells Treated in Vitro with ASS against Cells of Line CBA Mice

| Donor | Number of transplanted cells | Serum | Dilution | Recipient | No. of animals | Number of Foci in spleen | $t$ | $P$ |
|---|---|---|---|---|---|---|---|---|
| CBA | $2,5 \cdot 10^6$ | ASS, original | $10^{-1}$ | CBA | 8 | 1,2 (0—5) | | |
| » | $2,5 \cdot 10^6$ | " " | $10^{-3}$ | " | 4 | 2,0 (1—4) | | |
| » | $2,5 \cdot 10^6$ | " " | $10^{-4}$ | " | 8 | 7,4 (4—10) | 4,45 | 0,002 |
| CBA | $2,5 \cdot 10^6$ | ASS, exhausted with erythrocytes | $10^{-1}$ | CBA | 7 | 0 | | |
| » | $2,5 \cdot 10^6$ | Ditto | | | | | | |
| » | $2,5 \cdot 10^6$ | " | $10^{-3}$ | " | 7 | 1,3 (0—3) | | |
| | | | $10^{-4}$ | " | 7 | 7,1 (2—16) | 4,45 | 0,002 |
| CBA | $2,5 \cdot 10^6$ | NRS | $10^{-1}$ | CBA | 12 | 14,7 (6—23) | 0,874 | 0,5 |
| » | $2,5 \cdot 10^6$ | Hanks' solution | — | " | 11 | 17,0 (10—27)* | | |
| » | — | — | — | " | 11 | 0 | | |
| CBA | $2,5 \cdot 10^6$ | ASS, exhausted by erythrocytes and kidney cells | Undiluted | $F_1$ | 4 | 1,0 (0—2) | | |
| | — | | $10^{-1}$ | » | 6 | 1,8 (0—3) | | |
| » | $2,5 \cdot 10^6$ | Ditto | $10^{-3}$ | » | 4 | 2,5 (2—4) | | |
| » | $2,5 \cdot 10^6$ | " | $10^{-4}$ | » | 2 | 3,5 (3—4) | | |
| » | $2,5 \cdot 10^6$ | " | — | » | 8 | 10,0 (5—13) | | |
| » | $2,5 \cdot 10^6$ | Hanks's solution | — | — | 10 | 0,6 (0—3) | | |
| | — | — | | | | | | |

* Significance of difference calculated by dispersion analysis relative to the given value.

TABLE 2. Effect of ASS on Spleen Cells from Mice of Immunizing and Allogeneic Lines

| Group | Donor | Number of transplanted cells | Serum | Dilution of ASS | No. of $F_1$ recipients irradiated with 830 rad | Number of foci in spleen | $t$ | $P$ |
|---|---|---|---|---|---|---|---|---|
| 1-я | CBA | $2,5 \cdot 10^6$ | Anti-CBA ASS | $10^{-3}$ | 15 | 1,8±0,4 (0—5) | 2,34 | 0,05 |
| 2-я | » | $2,5 \cdot 10^6$ | Anti-C57BL ASS | $10^{-3}$ | 16 | 3,6±0,6 (1—9) | | |
| 3-я | » | $2,5 \cdot 10^6$ | Hanks's solution | — | 7 | 11,8±1,7 (8—19) | 8,25 | <0,01 |
| 4-я | — | — | — | — | 8 | 1,1±0,6 (0—3) | | |
| 5-я | C57BL | $2,5 \cdot 10^6$ | Anti-CBA ASS | $10^{-3}$ | 19 | 2,7±0,1 (0—8) | 0,9 | >0,25 |
| 6-я | » | $2,5 \cdot 10^6$ | Anti-C57BL ASS | $10^{-3}$ | 19 | 3,4±0,13(0—8) | | |
| 7-я | » | $2,5 \cdot 10^6$ | Hanks's solution | — | 12 | 12,5±2,04(5—26) | 4,94 | 0,001 |
| 8-я | — | — | — | — | 17 | 0,3±0,03(0—2) | | |

TABLE 3. Depression of Clone Formation under the Influence of Anti-CBA ASS Relative to Duration of Incubation (Transplantation of $2 \times 10^6$ spleen cells; dilution of ASS $10^{-3}$; donors CBA mice; recipients DBA mice irradiated with 830 rad)

| Time of treatment of serum (in min) | No. of animals | No. of foci in spleen | $t$ | $P$ |
|---|---|---|---|---|
| 5 | 10 | 6,5±0,27 (3—10) | 2,25 | 0,05 |
| 15 | 8 | 4,25±0,27 (2—7) | | |
| 30 | 6 | 3,5±0,2 (2—5) | 0,75 | 0,5 |
| 60 | 7 | 5,3±0,53 (1—9) | 0,8 | 0,5 |
| Hanks's solution | 6 | 17,1±0,36(15—20) | | |

the spleen cells with ASS in vitro led to inhibition of the formation of foci. Despite fluctuations in their number in individual animals, the decrease was obvious and statistically significant. A small decrease in the number of foci formed, not statistically significant, also occurred after treatment with NRS (Table 1).

Exhaustion of ASS with anti-CBA erythrocytes and with kidney cells of C57BL mice in order to exclude any possible effect of type-specific antibodies did not alter the effectiveness of the ASS (Table 1). The antisera were highly effective both in syngeneic and in allogeneic combinations (Table 2). However, when CBA mice were used as donor, anti-CBA ASS was more effective than anti-C57BL ASS, as statistical analysis confirmed (groups 1 and 2). There are, therefore, grounds for supposing that species-specific antibodies were present against mouse spleen cells of the immunizing line. However, in the other combination, when the donors were C57BL mice, the "syngeneity" of the ASS gave no advantage, for depression of clone formation was practically the same (groups 5 and 6). A definite class of sera with a marked syngeneic action evidently exists [12].

A discrepancy was noted between the cytotoxic titer of the ASS and its dilution which gave inhibition of clone formation. The same result has been observed in relation to bone marrow [6]. The difference was at least 2 orders of magnitude. During treatment of the cells in vitro the serum was inactivated and no complement was added. This excludes a cytotoxic effect in vitro. If, despite this, it is still considered that the effect of inhibition is connected with cytotoxic action (recipient's complement), it must be assumed that the stem cells possess increased ability to absorb antiserum or that they are much more sensitive than the other cells to the cytotoxic action of ASS. The results showed that the duration of contact between ASS and the cells in vitro within the range from 5 to 60 min had no significant effect on the results (Table 3). This also confirms the hypothesis that stem cells have a special affinity for antiserum.

The possibility is not ruled out that inhibition of the formation of foci is the result of the blast-transforming [7] action of ASS.

It has thus been found that ASS has a marked inhibitory action on the stem cells of hematopoietic tissue. This effect of ASS is exhibited in subcytotoxic concentrations after contact in vitro for the minimum of time. It must be assumed that ASS is not without its effects on the body, and that when it is used, its action on hematopoietic tissue must be taken into account.

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