during the first hour can be regarded as reliably established. It shows that electron-excited states which are deactivated by emission are present in the activated lymphocytes. In the writer's opinion another no less important fact is that the activated lymphocyte culture induces radiation in a culture not activated by optical contact, and, moreover, it does so very rapidly. As will be clear from Fig. 2, the two curves have a practically identical time course.

Taking all these circumstances into account and also having regard to opinions of other workers that electron-excited states play an important role in the course of vital processes in the cell [5, 6], the results described above can be interpreted as reflecting optical interaction between lymphocytes. The view expressed previously [1] that optical contact is a possible mechanism of nonspecific activation of lymphocytes during realization of the immunologic response at the cellular level is confirmed.

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# ROLE OF CELL SURFACE H-2 ANTIGENS IN REGULATING PROLIFERATION OF HEMATOPOIETIC STEM CELLS


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Transplantation antigens of a nonsyngeneic recipient can appear as "histocompatibility molecules" on the surface of a donor's hematopoietic stem cells (HSC), which facilitate their subsequent growth in the same recipient [2]. This suggests that transplantation antigens take part in intercellular interactions of HSC with their microenvironment that are essential for regulating HSC proliferation.

In the investigation described below proliferation of antigenically modulated HSC, transplanted into a syngeneic irradiated recipient, was studied.

## EXPERIMENTAL METHOD

C57BL/6 (subsequently abbreviated to B6) and (CBA × C57BL)$F_1$ mice (abbreviated to $CBF_1$), of both sexes, were used. HSC were determined by cloning in the spleen of irradiated mice [4]. To study induction of proliferation in resting HSC the "suicide" method with hydroxyurea (HU) was used. Normal or antigenically modulated HSC were injected in a dose of $6 \times 10^6$ to $25 \times 10^6$ bone marrow cells into irradiated (11-12 Gy) intermediate recipients. The spleen was removed from the latter 1-3 h later and the suspension of splenocytes was divided into two equal parts, one of which was incubated in medium 199 enriched with 2 mM L-glutamine and 5% embryonic calf serum, with 1 mg/ml (16 mM) HU at 37°C for 1 h; the other half was incu-


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TABLE 1. Sensitivity of Normal HSC of B6 Mice and HSC Temporarily without H-2 Antigens during First Hours after Transplantation into Irradiated Syngeneic Recipient (mean results of four experiments)

| Treatment | Duration of stay in intermediate recipient, h | "Suicide" with hydroxyurea, % |
|---|---|---|
| NMS[1] | 1 | 20±5.5 |
| CBA-a-B6+anti-γ | 1 | 0 |
| NMS | 2 | 28±5.3 |
| CBA-a-B6+anti-γ | 2 | 4±2 |
| NMS | 3 | 30±5.5 |
| CBA-a-B6+anti-γ | 3 | 40±6.4 |

Legend. Here and in Tables 2 and 3, NMS denotes normal mouse serum.

TABLE 2. Sensitivity of Normal and Antigenically Modulated HSC of $CBF_1$ Mice to HU during First Hours after Transplantation into Irradiated Syngeneic Recipient

| Expt. No. | Treatment | Duration of stay in interm. recip., h | HU | No. of colonies per spleen (M ± m) | "Suicide" with hydroxyurea, % |
|---|---|---|---|---|---|
| 1 | NMS | 1 1/2 | — | 29,7±5,6 | 27 |
| | | | + | 22,7±3,4 | |
| | CBA-a-B6 +anti-γ | 1 1/2 | — | 6,6±0,9 | 3 |
| | | | + | 6,4±0,9 | |
| | B6-a-CBA +anti-γ | 1 1/2 | — | 14,4±1,1 | 0 |
| | | | + | 17,7±4,1 | |
| | B6-a-CBA+CBA-a-B6 + anti-γ | 1 1/2 | — | 9,2±0,5 | 0 |
| | | | + | 10,8±0,8 | |
| 2 | NMS | 2 | — | 46,2±5,1 | 20 |
| | | | + | 37,0±2,1 | |
| | CBA-a-B6 +anti-γ | 2 | — | 19,8±2,3 | 6 |
| | | | + | 18,6±2,1 | |
| | B6-a-B6c-a-CBA + anti-γ | 2 | — | 31,4±4,3 | 4 |
| | | | + | 30,0±4,1 | |

TABLE 3. Sensitivity of Normal HSC of B6 Mice and HSC Antigenically Modulated Relative to the K- or D-Region of the H-2 Complex during First Hours after Transplantation into Irradiated Syngeneic Recipient

| Treatment | Duration of stay in intermediate recipient, h | HU | No. of colonies per spleen (M ± m) | "Suicide" with hydroxyurea, % |
|---|---|---|---|---|
| NMS | 1 | — | 23,0±1,2 | 16 |
| | | + | 19,4±2,0 | |
| CBA-a-B6, adsorbed with R101 + anti-γ[1] | 1 | — | 12,9±1,4 | 0 |
| | | + | 12,9±1,5 | |
| CBA-a-B6, adsorbed with 5R + anti-γ[2] | 1 | — | 10,2±1,1 | 8 |
| | | + | 9,4±1,3 | |

*HSC preserve only the K-region of the H-2 complex after such treatment.
†HSC preserve only the D-region of the H-2 complex after such treatment.

bated without HU. After the cells had been washed once they were injected (0.25 of a splenic equivalent) into irradiated recipients to determine the number of HSC in the suspensions. "Suicide" (S) was calculated by the equation:

$$S\ (\text{in } \%) = \frac{100 \times (\text{number of colonies without HU} - \text{number with HU})}{\text{Number of colonies without HU}}.$$

C57BL-anti-CBA (abbreviated to B6-a-CBA) and CBA-anti-C57BL (CBA-a-B6) antisera and rabbit serum against mouse γ-globulins (anti-γ) were prepared and characterized and the conditions for antigenic modulation of HSC were chosen as described previously [1]. Temporary deprivation of the cells of transplantation antigens was carried out by treating them with the corresponding antiserum without complement, and later with anti-γ-serum; complexes of H-2 antigens with antibodies and anti-antibodies "desquamate" from the surface of HSC after such treatment. To obtain antisera against the K- or D-regions of the H-2 complex, CBA-a-B6 serum was absorbed twice with spleen cells from B10·D2 (R101)(H-2 $K^bD^d$) or B10·A (5A)(H-2 $K^dD^b$) mice, which have a common D or K region respectively with B6 mice. After absorption with R101 or 5R cells the cytotoxicity of the sera against B6 cells was reduced very slightly (from 1:512 and more to 1:512-1:256), whereas cytotoxic activity against R101 or 5R cells disappeared or fell to a titer of 1:8. Unabsorbed sera, when incubated with HSC in a dilution of 1:4 without complement, inactivated 94% of HSC of B6 mice, but after absorption with cells of R101 or 5R recombinants, they inactivated 88-87% of HSC, which did not differ significantly from the activity of the unabsorbed serum.

## EXPERIMENTAL RESULTS

It was shown previously that HSC transplanted into an irradiated syngeneic recipient commenced the cycle after 1 h and a high proportion of them became sensitive to HU; antigenically modulated HSC, temporarily without transplantation antigens, did not enter the cycle until after 3 h.

The results (Table 1) are evidence that transplantation antigens are essential for the induction of proliferation in resting HSC. Normal interaction of the hematopoietic microenvironment of the irradiated recipient with HSC, as a result of which proliferation of the resting HSC is induced, is possible only if H-2 antigens are present on the surface of the HSC; these antigens reappear 3 h after antigenic modulation. Preservation of the unchanged structure of all transplantation antigens on the cell surface is necessary for successful regulation of HSC proliferation. If transplantation antigens of both parents or of either of them are removed from HSC of $DBF_1$ mice, these modulated cells cannot commence the cycle at times when normal HSC have already begun to proliferate after transplantation (Table 2). Not only all H-2 antigens must be present on the HSC for complete sensitivity to normal regulation, but these antigens must not be modified. Removal of antigens of only the K- or D-regions of the H-2 complex from the cell surface disturbs regulation, and HSC modified in this way cannot commence the cycle rapidly in an irradiated recipient (Table 3).

These results are the first experimental proof of the role of H-2 antigens of HSC in the regulation of histogenesis in myeloid tissue. It is easy to imagine that the evolutionary development of such mechanisms led to the appearance of the recently discovered H-2-restriction during interaction between lymphoid cells [3].

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