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SECRETION OF SUPPRESSOR FACTORS BY MOUSE LYMPHOCYTES ON CONTACT WITH SYNGENEIC AND XENOGENEIC RED CELLS

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During incubation of spleen cells of immune mice *in vitro* with syngeneic and xenogeneic red cells a factor (or factors) with immunosuppressive activity is secreted into the medium. Secretion of the suppressor factor by spleen cells of nonimmune mice takes place only on contact with xenogeneic red cells.

KEY WORDS: *immune response; immunosuppression.*

Evidence has now been obtained that the immune response is under the control of T cells and, in particular, through the secretion of humoral factors [1, 8]. It has been shown, for instance, that on incubation of human T lymphocytes (strain MOLT) with sheep's red cells secretion of a factor (or factors) reducing the number of antibody-forming cells (AFC) in a culture of spleen cells of mice immunized with sheep's red cells *in vitro* [6] occurs. Activated mouse lymphocytes, when incubated with antigen, secrete a dialyzable factor which enhances the immune response [5, 7].

In this investigation the ability of mouse lymphocytes to secrete an immunosuppressive factor on contact with syngeneic and xenogeneic red cells was studied.

EXPERIMENTAL METHOD

(CBA × C57BL)F_1 mice were immunized intravenously with $2 \cdot 10^8$-$3 \cdot 10^8$ sheep's red cells. Three days later some of them were killed to obtain the test factor. Experimental immunized

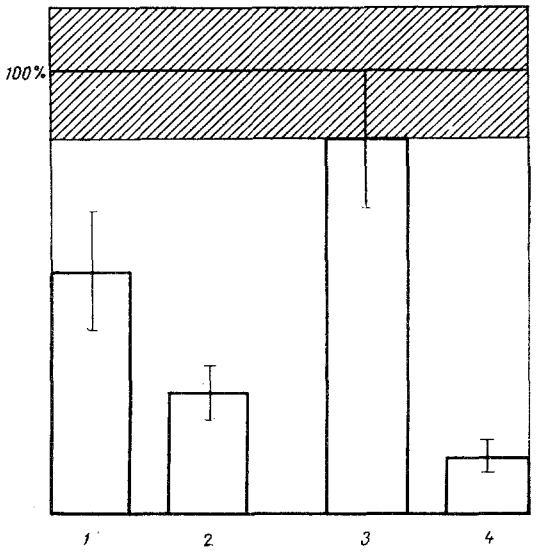

Fig. 1. Effect of supernatants obtained on contact between spleen cells and red cells on immune response: 1) nonimmune mouse lymphocytes + sheep's red cells; 2) immune mouse lymphocytes + sheep's red cells; 3) nonimmune mouse lymphocytes + syngeneic red cells; 4) immune mouse lymphocytes + syngeneic red cells. Continuous line in shaded zone indicates PFC level in control (in mice immunized with sheep's red cells), taken as 100%. Shaded zone indicates 95% confidence limits.

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TABLE 1. Effect of Factor Secreted after Contact between Lymphocytes and Red Cells on Primary Immune Response

Spleen cells	Red cells	Preliminary centrifugation (300 g)	Number of animals	Number of PFC in spleen (X_g and 95% confidence limits)
Immune	Mouse	+	10	1148 (807—1633)
"	Sheep	+	5	2549 (1099—5912)
"	Mouse	—	5	2003 (1146—3510)
"	Sheep*	—	5	10870 (5332—22190)
Immune lymphocytes without red cells	—	+	10	2761 (1557—4896)
	—	+	5	10810 (4743—24680)
	—	—	10	11320 (7973—16080)

*Suspension contained its "own" red cells.

mice were given an injection of 0.5 ml of the supernatant containing the test factor immediately after it had been obtained. The experimental mice were killed 16-18 h after the injection of the factor and the number of plaque-forming cells (PFC) in the spleen was determined by the methods of Jerne [4] and Cunningham [3]. The supernatant containing the test factor was obtained as follows. Equal volumes of spleen cells ($25 \cdot 10^6$/ml) and a 0.5% suspension of red cells were mixed and incubated for 2 h at 37°C with or without preliminary centrifugation (300g) for 10 min. At the end of incubation the mixture was again centrifuged under the same conditions. Immune spleen cells previously freed from red cells in a Ficollurotrast density gradient (1.077 g/cm^3) [2] were centrifuged and incubated for 2 h at 37°C and the supernatant was injected into one group of control mice; intact mice served as the other control group.

EXPERIMENTAL RESULTS

The results given in Table 1 show that injection of the supernatant obtained after incubation of a previously centrifuged mixture of immune mice lymphocytes and syngeneic red cells reduced the number of PFC in the spleen from 11,320 (control) to 1148 (experiment; $P < 0.001$). The number of PFC in the spleen was affected by injection of the supernatant obtained after incubation of the centrifuged mixture of immune mouse spleen cells with sheep's red cells (the number of PFC was 2549; $P < 0.01$) and with mouse red cells without preliminary centrifugation (number of PFC 2003; $P < 0.01$). Injection of the supernatant obtained after incubation of immune spleen cells with sheep's red cells without preliminary centrifugation did not affect the number of PFC in the spleen of the immune mice. Secretion of the suppressor factor also took place on incubation of previously centrifuged immune spleen cells (number of PFC 2671; $P < 0.01$). However, this mixture contained syngeneic splenic red cells (ratio of red cells to lymphocytes 1:2-1:1), whereas on incubation of spleen cells from which the red cells had been removed no suppressor factors were secreted. The results on the secretion of suppressor factors on contact between immune lymphocytes and syngeneic red cells were confirmed in other experiments (in which the PSC were counted by Cunningham's method). These experiments showed that the number of PFC in the spleen of the immune mice fell from 66 ± 6.7 in the control to 22 ± 2.6 per 10^6 spleen cells in the experiments ($P < 0.005$). The factor secreted after contact between immune lymphocytes and sheep's red cells also had an inhibitory action (33 ± 9.7; $P < 0.025$).

The results thus indicate that a factor inhibiting the immune response is secreted during contact between immune spleen cells and syngeneic red cells.

The suppressor factor also was secreted on contact between immune spleen cells and sheep's red cells, acting in this case as an antigen. Ability to secrete substances inhibiting the immune response has been found to be a property not only of immune but also of nonimmune lymphocytes [6, 8].

In the next series of experiments an attempt was made to discover whether suppressor factors are secreted on contact between nonimmune spleen cells and syngeneic and xenogeneic red cells. In this series of experiments a mixture of red cells and lymphocytes was centrifuged for 10 min at 300g and then treated as described in "Experimental Method." The results are given in Fig. 1 and show that contact between nonimmune spleen cells and syngeneic red cells did not lead to the secretion of suppressor factors. On the other hand, on contact between nonimmune spleen cells and sheep's red cells, these factors were secreted.

Comparison of the results suggests that only immune mouse lymphocytes, on contact with syngeneic red cells, can secrete the suppressor factor which probably participates in the regulation of the immune response. Secretion of suppressor factors observed on contact between immune lymphocytes and cells of the same genotypes may play a role in the maintenance of the normal function of the T and B systems of immunity. A disturbance of this mechanism may perhaps play a role in the development of autoimmune diseases.

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IMMUNOCOMPETENCE OF LYMPHOCYTES FROM PREGNANT MICE STUDIED IN GRAFT VERSUS HOST REACTION

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The ability of lymphocytes taken during the second trimester from C57BL/6 mice mated with CBA males to induce the graft versus host reaction in (CBA × C57BL/6)F_1 hybrids was weaker than that of cells both of virgin donors and of mice pregnant after syngeneic mating. This was reflected in lengthening of the life span of the experimental recipients and weakening of inhibition of endogenous colony formation in the spleen of sublethally irradiated hybrids. This ability was restored at the end of pregnancy and in some experiments it actually exceeded the control.

KEY WORDS: *graft versus host reaction; pregnancy.*

There is no question about the important role of the mother's cellular immunity in preventing immunological conflict with her own fetus [1, 2, 5]. One of the main manifestations of the immunological activity of lymphocytes is their ability to induce a graft versus host reaction (GVHR). The writers are aware of only a few investigations in which this test was used to study cellular immunity in pregnancy. With the local GVHR as model, a nonspecific lowering of immunological reactivity has been demonstrated in the second half of pregnancy incompatible for the H-2 complex, but not syngeneic, in mice [9]. In experiments using systemic and local GVHR, evidence was obtained of an increase in sensitization of maternal lymphocytes to transplantation antigens of paternal origin [8, 10].

In the investigation described below the ability of lymphocytes of the spleen and lymph nodes of the parents to induce a systemic GVHR in their offspring (F_1 hybrids) was studied.

EXPERIMENTAL METHOD

Experiments were carried out on virgin female C57BL/6 mice and (CBA × C57BL/6)F_1 hybrids of both sexes. The mice were obtained from the nursery of inbred animals, Academy of

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