tissue cultures distant interaction takes place and is expressed as the repetition of the morphological features of the cytopathological process induced in one of the cultures by means of viruses or mercuric chloride, in the other intact tissue culture, or in other words, a "mirror" CPE takes place.

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ROLE OF GENETIC DIFFERENCES BETWEEN MOTHER AND FETUSES IN THE DEVELOPMENT OF A GRAFT VERSUS HOST REACTION INDUCED IN THE MOTHER

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Development of the graft versus host reaction (GVHR) was studied in female (CBA×C57BL/6)F_1 mice during pregnancy, and after birth or the day before mating with syngeneic, semisyngeneic, and allogeneic males. The development and outcome of the GVHR in the female mice was shown to depend on genetic differences between the donors of transplanted lymphocytes and the fetuses and also on the time of induction of the GVHR. If lymphocytes from C57BL/6 mice were injected into (CBA×C57BL/6)F_1 females after parturition or on the day before mating with males of the parental CBA line, pregnancy led to enhancement of the GVHR; if lymphocytes were injected during pregnancy, an increase in resistance to the BVHR was observed. In the case of mating with males of the contralateral parental line C57BL/6 (syngeneic with respect to the lymphocyte donors) pregnancy did not affect the development of the GVHR regardless of the time when the cells were injected.

KEY WORDS: immunopathology of pregnancy; mother–fetus; graft versus host reaction; genetic differences.

During pregnancy the maternal immune system is exposed to the action of different cells and subcellular factors carrying genetic information of the fetus. The biological signifi-

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cance of this exposure is to ensure immunologic control of the mother over embryogenesis [1, 2, 12]. There is also evidence that the fetus exerts its influence on the specific immunologic reactivity of the mother [8]. However, it is not known whether the fetus can exert its specific influence on the development of pathological processes in the maternal immune system and, if it can, whether this influence depends on genetic differences between mother and fetus. To study this problem experimentally, the graft versus host reaction (GVHR) was used, for it can be regarded as model of autoimmune disturbances [4, 7, 11].

The object of this investigation was to study the development of a systemic or local GVHR induced in female F_1 hybrid mice during pregnancy, after giving birth, or on the day before mating with syngeneic, semisyngeneic, and allogeneic males. In this particular experimental model, the F_1 hybrid females were unable, for genetic reasons, to react immunologically against F_2 hybrid fetuses obtained as a result of direct or reciprocal crossing.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature mice obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR. (CBA×C57BL/6)F_1 ($H\text{-}2^k/H\text{-}2^b$) females were crossed with (CBA×C57BL/6)F_1, CBA ($H\text{-}2^k$), C57BL/6 ($H\text{-}2^b$), or DBA/2 ($H\text{-}2^d$) males. A systemic GVHR was induced in the females 2-4 days before parturition (experiments of series I), 7-10 days before mating (series II), or 2-3 days after parturition (series III) by intravenous injection of 25-60 million living spleen and lymph node cells from C57BL/6 mice. Fuller details of the method used to induce the GVHR were described previously [5]. In the experiments of series IV a local GVHR was induced in normal females 2-3 days after parturition by the method of Ford et al. [10] in the modification [6], in the popliteal lymph nodes. On the 6th day after induction of the GVHR the popliteal lymph nodes were removed, dehydrated in acetone, dried in air, and weighed, after which the ratio of the weight of the stimulated lymph nodes to the weight of the unstimulated (contralateral) lymph nodes was calculated. In each series of experiments a systemic and local GVHR also was induced in control unmated mice. The index of significance of differences between the various groups was determined by Fisher's method for a fourfold table [3].

EXPERIMENTAL RESULTS

As Table 1 shows, in most females becoming pregnant as a result of mating with syngeneic males (direct cross), the GVHR induced in them a few days before giving birth (series I) did not end in death (group 1). In the control (group 5), after transplantation of the same dose of lymphocytes, all recipients died from the GVHR. In mice becoming pregnant as a result of reciprocal crossing with males of the parental line C57BL/6 (group 3) the mortality from the GVHR induced during pregnancy was almost the same as in the control. Reciprocal crossing with males of the contralateral parental line CBA (group 2) led to an increase in the resistance of the pregnant mice to GVHR. When the females were crossed with males of the third (unrelated) line DBA/2 some animals did not die from the GVHR induced during pregnancy, but these differences (group 4) compared with the control were at a low level of statistical significance. Consequently, in cases when males genetically different from the donors of the transplanted lymphocytes were used for mating, pregnancy led to weakening of the GVHR.

Completely opposite results were obtained as a result of injection of 25 million lymphocytes from C57BL/6 mice into the females 7-10 days before pregnancy (series II). In the intact control mice (group 9) this dose of cells did not cause death within the period of observation. In females becoming pregnant 7-10 days after induction of the GVHR, pregnancy ended with normal birth, but some of the recipients died later from transplantation sickness. The highest mortality ($P = 0.025$) was observed among females mated with CBA males (group 6). In that case, if the GVHR was induced by C57BL/6 lymphocytes in a dose of 60 million, all the experimental females crossed with CBA or C57BL/6 males died during pregnancy.

A study of the GVHR induced in females 2-3 days after parturition showed that transplantation of lymphocytes from C57BL/6 mice in a dose of 25 million led to death of the recipients only if mated with CBA males (series III; group 10). However, these differences compared with the control were not statistically significant, possibly on account of the low sensitivity of the systemic GVHR technique. To obtain more accurate data, in the next series (IV) of experiments an attempt was made to compare the development of the local GVHR in the popliteal lymph nodes of females which had and had not littered. As Table 2 shows, the sensitivity of females mated with C57BL/6 males was unchanged after parturition (group 2) com-

TABLE 1. Development of Systemic Graft Versus Host Reaction Induced by Lymphocytes from C57BL/6 Mice in (CBA×C57BL/6)F_1 Females Mated with Males of Different Lines

Series of expt.	Time of induction of GVHR	Group of animals	Females	Males	No. of mice in experim.	No. of lymphocytes millions	No. of mice dying in 100 days	
							absolute	%
I	2-3 days before parturition	1-	Experiment	F_1	23	60	8*	34,7
		2-	»	CBA	18	60	6*	33,3
		3-	»	C57BL/6	20	60	16*	80
		4-	»	DBA/2	17	60	10	58,8
		5-	Control	—	15	60	15	100
II	7-10 days before mating	6-	Experiment	CBA	34	25	12*	35,3
		7-	»	C57BL/6	28	25	2	7,1
		8-	»	DBA/2	20	25	1	5
		9-	Control	—	15	25	—	—
III	2-3 days after parturition	10-	Experiment	CBA	20	25	4	20
		11-	»	C57BL/6	18	25	—	—
		12-	»	DBA/2	15	25	—	—
		13-	Control	—	15	25	—	—

*Differences between experiment and control statistically significant ($P \leqslant 0.025$).

TABLE 2. Relative Weight of Popliteal Lymph Nodes After Induction of Local Graft Versus Host Reaction by Lymphocytes from C57BL/6 Mice in Littered and Nonpregnant (CBA×C57BL/6)F_1 Females

Series of expt.	Females	Males	No. of mice	Ratio of wt. of stimulated popliteal lymph node to wt. of unstimulated lymph node	
				arithmetic mean and limits of variations	P
I	Experim.	CBA	11	4,42 (2,0—7,7)	<0,025
II	»	C57BL/6	12	2,89 (2,0—5,0)	>0,025
III	Control	—	12	2,87 (1,6—5,0)	—

pared with the control (group 3), whereas after mating with CBA males, intensification of the local GVHR was observed in the females which had littered (group 1).

It can be concluded on the basis of these results that genetic differences between mother and fetuses play an essential role in the onset and development of certain pathological reactions in the maternal immune system of the GVHR type. In the experimental model used in the present experiments, F_1 hybrids for genetic reasons could not react immunologically against F_2 hybrids obtained as a result of direct or reciprocal crosses. It can therefore be suggested that specific changes in the immune system of the mother arising during pregnancy and persisting after birth are due to the action of immunocompetent cells of the fetuses on the mother. A similar suggestion was expressed previously by other workers [8, 9]. In some cases fetal lymphocytes have been found in the mother's body [9], but the immunocompetence of these cells was not proved.

The possibility cannot be ruled out that lymphocytes of embryos or of the fetal part of the trophoblast, penetrating into the maternal circulation, not only contribute to a decrease in specific reactivity against the fetuses [8], but also participate in the maintenance of homeostasis of the maternal immune system, by inactivating cells which appear during pregnancy and against which the mother, for genetic or other reasons, cannot react. This hypothesis is indirectly confirmed by the fact, which the writers discovered, that the GVHR induced in females during pregnancy is inhibited only if the donors of the transplanted lymphocytes differ genetically from the fetuses and that a graft versus host reaction between them is theoretically possible.

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INTERFERON PRODUCTION DURING INTERACTION BETWEEN LYMPHOCYTES AND TARGET L-CELLS IN DIFFERENT PHASES OF THE MITOTIC CYCLE

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Interferon is found in the culture medium after interaction between target L-cells synchronized (in the G_1 and S) and unsynchronized with lymphocytes (intact, immune, and nonimmune, treated with actinomycin D). On interaction between immune lymphocytes and L-cells in the G_1 phase, interferon production visibly commences after 30 min (25 units/ml), reaches a maximum after 10 h (75 units/ml), and then falls slightly to 48 h (65 units/ml). Interferon production during interaction between immune lymphocytes and L-cells in the G_1 phase correlates with the cytotoxic action of the lymphocytes on these cells.

KEY WORDS: interferon; lymphocytes; target cells; cell cycle.

During interaction between immune lymphocytes and target cells, interferon is found in the culture medium [7, 10]. Interferon production *in vivo* was demonstrated previously in the writers' laboratory in mice in response to injection of antigenically foreign materials [5]. It was suggested that interferon production during interaction between immune lymphocytes and target cells is connected with recognition of histocompatibility antigens by the lymphocytes. In that case, the intensity and rate of interferon production may differ depending on the phase of the cell cycle in which the target cells are found, for histocompatibility antigens are known to be expressed to different degrees on the cell surface: maximally in the G_1 phase and minimally in the S phase [8, 9].

This paper gives data on the investigation of interferon production during interaction between synchronized and unsynchronized target L-cells with immune, intact, and actinomycin D-treated lymphocytes.

EXPERIMENTAL METHOD

Synchronized and unsynchronized transplantable mouse L fibroblasts from C3H ($H\text{-}2^k$) mice were used as target cells. Synchronization was carried out by the addition of an excess of thymidine (2 mg/ml) [12]. The mean degree of synchronization was 80-85%. Parameters of the mitotic cycle of the L-cells were determined from the curve of labeled mitoses at the 50%

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