DEVELOPMENT OF THE GRAFT VERSUS HOST REACTION AND ITS EFFECT ON PREGNANCY IN MICE AFTER HEPARIN ADMINISTRATION

E. A. Fedosov

UDC 578.089.843:612.6-017.1

The effect of heparin on the graft versus host reaction (GVHR) was studied in mice and the character of development of pregnancy in females surviving after the GVHR was noted. Preliminary injection of heparin into the recipients prevented their death from the GVHR or lengthened their life span. After injection of heparin into the donors or its addition to the transplanted cells, the GVHR was intensified. In mice surviving after the GVHR as a result of heparin administration, in 60-100% of cases abortion or intrauterine death of the fetus was observed during pregnancy (3-6 months after transplantation of the cells). When these females were again mated pathological pregnancies were observed less frequently, but some of the progeny developed runt disease. No such disturbances of pregnancy were observed in mice receiving heparin alone or surviving after transplantation of lymphocytes alone. Pregnancy enhanced the GVHR induced previously in females after injection of heparin.

KEY WORDS: *graft versus host reaction; heparin; pathology of pregnancy.*

The development of a graft versus host reaction (GVHR) is the main obstacle to the successful use of transplantation of hematopoietic tissue for the treatment of radiation sickness and of immunodeficient states [6, 9]. Clinical and experimental investigations have also yielded evidence of the role of this reaction in the development of immunologic conflict in

TABLE 1. Effect of Heparin on Development of the GVHR in F_1 Hybrids after Transplantation of Lymphocytes from C57BL/6 Mice

Expt. No.	Group of animals	Number of cells, millions	Administration of heparin: strain of mice	Administration of heparin: time (before or after induction of GVHR)	Number of mice in group	Number of mice dying from GVHR within 100 days	Life span of dying mice, days: arithmetic mean and limits of variation	Life span of dying mice, days: P
1	1	60	F_1	Before	10	10	28,7 (18—42)	<0,025
	2	60	—	—	12	11	16,3 (15—24)	—
2	3	40	F_1	Before	15	2 (P<0,025*)	54,5 (44—65)	>0,025
	4	40	—	—	12	11	28,5 (14—56)	—
3	5	30	F_1	Before	20	0 (P<0,025*)		
	6	30	—	—	20	9	28,4 (16—41)	
4	7	60	F_1	After	25	24	20,0 (17—24)	>0,025
	8	60	—	—	14	14	17,8 (16—25)	—
5	9	40	C57BL/6	Before	15	14	18,5 (14—26)	<0,025
	10	40	—	—	15	13	31,4 (16—51)	—
	11	40	C57BL/6	Before†	15	13	17,2 (15—22)	<0,025
6	12	40	C57Bl/6	Before‡	15	14	20,1 (16—24)	>0,025
	13	40	—	—	15	12	27,4 (18—40)	—

*P shown only if difference between experimental and control series is significant.
†Heparin added to cell suspension.
‡Suspension washed to remove heparin.

Department of Microbiology, Smolensk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 3, pp. 312-315, March, 1977. Original article submitted January 22, 1976.

TABLE 2. Development of Pregnancy and GVHR in (CBA × C57BL/6)F_1 Mice Surviving after Administration of Heparin and Translantation of C57BL/6 Lymphocytes

Group of animals	Number of cells, millions	Administration of heparin	Number of mice in group	Number of mice becoming pregnant	Outcome of pregnancy (number of mice): died before parturition	abortions, stillbirths, non-occurrence of parturition	normal pregnancy	Total number of mice under observation after pregnancy	Number of mice dying within 8 months after pregnancy and parturition	P
1	40	+	13	11	2	9	—	6	5	0,025
2	30	+	20	18	1	10	7	14	10	0,025
3	30	—	11	10	—	—	10	10	2	—
4	—	+	15	15	—	—	15	15	—	

the mother–fetus system [5, 7-9]. Clarification of the mechanism of onset of the GVHR and the search for methods of its prevention are among the important tasks in transplantation immunology. Studies along these lines have been carried out with the use of heparin, which is known to influence several mechanisms of immunity: It weakens some reactions of cellular and humoral immunity [1, 4], inhibits interferon synthesis [3], and depresses the development of metastases of some tumors [11].

In this investigation the development of the GVHR was studied after administration of heparin to donors or recipients and also after treatment of donors' lymphocytes with heparin *in vitro*. The effect of the GVHR on pregnancy was studied in mice surviving after heparin treatment.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57BL/6)F_1 and C57BL/6 mice, obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR. To produce the GVHR, female F_1 hybrids were given an intravenous injection of a suspension containing 30-60 million living lymphocytes from C57BL/6 mice. The suspension was prepared by the method described previously [7]. Batches of donors and recipients received daily intravenous injections of 10 units heparin (Richter, Hungary) for 7 days before injection of the cell suspension. Some hybrids were given 10-50 units of heparin for 7 days after induction of the GVHR. In one series of experiments heparin was added to a suspension of lymphocytes from normal donors 30 min before its injection into normal hybrids (in a dose of 10 units per recipient). Part of this suspension was washed 3 times with medium No. 199 to remove the heparin. Mice receiving heparin or lymphocytes from normal donors only acted as the control. Females surviving after the GVHR were mated with syngeneic normal males. Fisher's method for a fourfold table was used to determine the degree of significance of differences between the experimental and control series.

EXPERIMENTAL RESULTS

As Table 1 shows, administration of heparin to the recipients on the day before transplantation of the cells led to an increase in the resistance of the mice to the GVHR (experiments 1-3). Depending on the dose of the cells this increase was expressed either as an increase in the life span (group 1) compared with the control (group 2; $P < 0.025$) or as complete prevention of mortality (groups 3 and 5). Administration of heparin to the recipients from the first day after induction of the GVHR (experiment 4) caused only a very small (not significant) increase in their life span (group 7).

In the next experiments the effect of heparin was studied on the ability of the donors' cells to induce the GVHR in normal hybrids (experiments 5 and 6). Injection of heparin into the donors on the day before cells were taken from them led to an increase in the severity of

the GVHR and shortening of the life span (group 9) compared with the control (group 10; $P < 0.025$). Similar results were obtained when heparin was added to the cell suspension before injection (group 11). After washing the suspension to remove heparin, the increased ability of the cells to induce the GVHR was somewhat reduced (group 12) and the results approximated those in the control (group 13).

Females recovering from the GVHR and also those receiving heparin alone were mated with males 3-6 months after the reactions. All females which survived after the GVHR were healthy during the mating period and in their external appearance were indistinguishable from the controls. The diagnosis of pregnancy was made on the basis of the increase in body weight, certain external manifestations, and palpation of the embryos through the abdominal wall. The results of observations on the development and outcome of pregnancy are given in Table 2.

Eleven of the 13 females in group 1 and 18 of the 20 females in group 2 became pregnant. In all pregnant mice of group 1 and in 11 of the 18 mice in group 2 pregnancy was abnormal. Three females dies before parturition and fetuses which had died at different stages of development were found at autopsy: Some embryos were well developed and showed signs only of maceration, whereas in other parts of the uterus the fetuses had been absorbed. Abortions or stillbirths were found in 17 mice. Three females with uterine hemorrhage were killed when their state was satisfactory and, besides dying and decomposed fetuses, 2 or 3 living embryos also were found in their uterus. In two females killed at the presumptive time of parturition, when they showed no evidence of disease, large abscesses were found in the uterus instead of a fetus. In another mouse an abscess was opened through the abdominal wall, after which the animal recovered and only died 7 months later. In nine females some embryos survived after abortion and sometime later they gave birth to 1-3 viable mice. In some of these females abortions also occurred during the 2nd and 3rd pregnancies, but the number of young mice born increased with each successive pregnancy. Some of the newborn animals died during the first days of life (14 of 48) and 9 died at the age of a few months. Their lymphoid organs were atrophied.

Most of the experimental females died. In group 1, 5 of the 6 mice surviving one or more pregnancies died in the course of 8 months; in group 2 10 of the 14 mice died. At autopsy they were found to have marked atrophy of the spleen, thymus, and lymph nodes. In the control (group 3: GVHR without preliminary administration of heparin) 2 of the 10 mice died during this period ($P = 0.025$). These results indicate that pregnancy aggravates the GVHR induced after injection of heparin into the recipients. In mice not receiving heparin but surviving after GVHR and also in mice receiving heparin alone (groups 3 and 4) no disturbances were observed in the course of pregnancy.

Heparin can thus weaken or enhance the GVHR. After preliminary treatment of the recipients with heparin conditions are evidently created for long persistence of the donor's cells (tolerance?), which under these conditions cannot react effectively to the host's tissues, but during pregnancy they are able to cause death of the fetuses, possibly as the result of a reaction to embryonic antigens. The possibility likewise cannot be ruled out that as a result of their reaction to embryonic antigens the donor's cells lose their tolerance to the host's tissues, and after pregnancy the GVHR is enhanced. Incidentally a similar pathology of pregnancy has also been observed after induction of the GVHR during the first days of pregnancy or a few days before its beginning [2, 5].

The mechanism of action of heparin and also the cause of its different effects after administration to donors or recipients are not clear. Perhaps under the influence of heparin the lymphocytes become more resistant to the reception of foreign genetic information and this may increase the chances of death of both the recipient's and the donor's cells. Evidence in support of this hypothesis is given by data showing the inhibitory action of heparin on the activity of reverse transcriptase [10], to which a role is ascribed in the reactions of immunity. The view has also been expressed that heparin affects differentiation processes taking place in the lymphocytes after contact with the antigen [1].

LITERATURE CITED

1. B. D. Brondz and A. S. Snegireva, Tsitologiya, No. 1, 74 (1973).
2. V. S. Dukova, A. S. Shevelev, and E. A. Fedosov, Byull. Éksp. Biol. Med., No. 9, 68 (1975).
3. L. M. Minkevich, L. V. Zhdanova, T. G. Orlova, et al., Vopr. Virusol., No. 4, 401 (1972).
4. R. V. Petrov, A. N. Cheredeev, and A. A. Gorokhov, in: Problems in Radiation Immunology and Microbiology [in Russian], Moscow (1972), pp. 13-14.

5. E. A. Fedosov, in: Problems in Radiation Immunity and Microbiology [in Russian], Moscow (1972), pp. 127-129.
6. E. A. Fedosov, Zh. Mikrobiol., No. 10, 87 (1974).
7. E. A. Fedosov, Dokl. Akad. Nauk SSSR, 222, 472 (1975).
8. A. E. Beer, R. S. Billingham, and S. L. Yang, J. Exp. Med., 135, 808 (1972).
9. R. A. Gatti, J. H. Kersey, E. Y. Yunis, et al., Prog. Clin. Path., 5, 1 (1973).
10. B. L. Kiehl, P. B. Stoff, A. C. Huang, et al., J. Nat. Cancer Inst. USA, 51, 1705 (1973).
11. I. A. Kiricuta, C. Todorutiu, T. Muresian, et al., Cancer (Philadelphia), 31, 1392 (1973).

SERUM ANTIBODIES AGAINST HEART VALVE FIBROBLASTS IN PATIENTS WITH RHEUMATIC FEVER

T. A. Danilova, N. M. Fedorova, and I. I. Rassokhina

UDC 616-002.77-07:616.126.3-008.9-097.5-078

Antibodies reacting in immunofluorescence tests with human and bovine heart valve fibroblasts were isolated from the serum of patients with rheumatic fever by means of an immunosorbent prepared from human heart valve tissues. The antibodies did not react with fibroblasts of the interstitial connective tissue of the myocardium. Fibroblasts of the myocardium and valves are evidently antigenically different, whereas fibroblasts of human and bovine heart valves have common antigens.

KEY WORDS: *rheumatic fever; fibroblasts; antibodies.*

Deposits of bound immunoglobulins have been found [7, 8] in connective-tissue structures of the myocardium and heart valves of patients with rheumatic fever. However, when the sera were studied by the immunofluorescence method, no circulating antibodies against connective tissue antigens were found [9, 13]. Previous investigations showed that the sera of patients with rheumatic fever react with cells of the interstitial connective tissue of bovine heart and from the heart of other animals [1]. Antibodies reacting with fibroblasts of various bovine organs, including heart valve fibroblasts, have been isolated by means of immunosorbents containing bovine connective tissue antigens from the sera of patients with rheumatic fever, but the antibodies thus obtained did not react with the connective tissue components of human heart [2].

The object of this investigation was to isolate antibodies from the sera of patients with rheumatic fever with the aid of an immunosorbent prepared from human heart valve tissue and to investigate them by the immunofluorescence method on sections from bovine and human heart valve and myocardial tissues.

EXPERIMENTAL METHOD

In order to isolate antibodies against human heart valve connective tissue antigens an immunosorbent was prepared from the valve tissues of healthy persons who had died from injury. The material was carefully homogenized in a tissue microblender at 4°C. The resulting homogenate was washed 4 or 5 times with 0.85% NaCl solution. Mechanical mincing of the homogenate and subsequent washing were repeated 4 or 5 times. The homogenate was then treated with 0.1 M glycine—HCl buffer, pH 2.8, and washed with 0.2 M phosphate buffer, pH 7.2, until protein disappeared from the supernatant.

Laboratory of Streptococcal Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR. Institute of Rheumatism, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 83, No. 3, pp. 315-317, March, 1977. Original article submitted June 29, 1976.