EFFECT OF LYMPHOCYTES SENSITIZED TO KIDNEY ANTIGENS, INJECTED INTO FEMALE MICE, ON RENAL FUNCTION OF THEIR PROGENY

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A model of autoimmune nephrosonephritis in CBA mice has been developed in recent years [1] and the existence of a link between the kidneys of mother and fetuses (progeny) has been established in it [2, 3]. Immunologic factors have been shown to play a role in the processes of interaction between the homonymous organs of mother and fetus [4].

The object of this investigation was a comparative study of neonatal kidneys depending on the time of injection of lymphocytes from syngeneic donors, sensitized to kidney antigens, into their mothers during pregnancy and before mating.

EXPERIMENTAL METHOD

Altogether 150 female CBA mice were used, of which 50 were the recipients and 100 were donors of lymphocytes. Nephrosonephritis was induced as a first step in 50 of the donors [1] and the other 50 donors were intact. A suspension of lymphocytes was obtained from the mesenteric lymph nodes of the donor mice. Sensitization of the lymphocytes to kidney antigen was determined by the macrophage migration inhibition test (MMIT). The method of obtaining the suspension of lymphocytes and of carrying out the MMIT was described previously [4]. Altogether 39 mice were investigated by this method, 19 with nephrosonephritis and 20 controls.

The recipient mice, divided into groups with three or four animals in each group, were given intraperitoneal injections of lymphocytes from donors with nephrosonephritis (experimental group) and from healthy donors (control group) at different times of pregnancy (10th-18th days) and one month before mating, in doses of 15 or 35 million cells in 0.5 ml Hanks's solution and in nutrient medium No. 199.

At the end of pregnancy the young were collected from all the mothers. The total number of newborn mice was 282: 147 in the experimental group and 135 in the control. The newborn mice were weighed, the relative weights of the kidneys and other organs (liver, heart, lungs, and spleen) was determined, and the kidneys were studied histologically and morphometrically. The significance of differences was determined by the Fisher–Student method. The following number of measurements of kidney structures was made: the diameter of the renal corpuscles and of the glomeruli – 502 experimental and 527 control; diameter of the proximal convoluted tubules and of their lumen – 762 experimental and 902 control; the height and width of the cells of the convoluted tubules – 1288 experimental and 1491 control; the diameter of the nuclei – 1692 experimental and 2039 control.

For histological analysis the kidneys were fixed in Bouin's fluid. Sections 4 μ thick were stained with Carazzi's hematoxylin and counterstained with eosin and by Schiff's reagent by Hotchkiss' method.

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TABLE 1. Dimensions of Structures of the Nephron of Newborn CBA Mice whose Mothers Received Injections of Lymphocytes from Syngeneic Donors with Diseased Kidneys and from Healthy Donors

Dose of lymphocytes, millions	Time of injection	Series of experiments	Diameter of renal corpuscles	Diameter of glomeruli	Diameter of convoluted tubules	Diameter of lumen of tubules	Height of cells of tubules	Width of cells of tubules	Diameter of nuclei of tubules
15	before conception	I	48,9±1,5	40,6±1,4	31,1±0,9	14,4±1,9	10,0±0,14	10,9±0,17	5,24±0,02
			50,2±1,3	43,0±1,1	31,7±1,9	16,9±0,9	9,30±0,15	9,40±0,13	5,10±0,09
		P	>0,05	>0,05	>0,05	>0,05	0,001	<0,001	<0,05
35	" "	II	50,7±1,3	43,3±1,2	33,6±0,9	14,0±0,7	11,2±0,19	11,2±0,22	5,24±0,03
			50,8±1,4	44,2±1,1	32,7±1,3	17,2±0,4	9,42±0,17	9,8±0,15	5,12±0,03
		P	>0,05	>0,05	>0,05	0,0001	<0,001	<0,001	<0,05
35	10th day of pregnancy	III	48,2±1,3	42,2±1,2	33,1±0,8	15,1±0,7	11,7±0,18	11,4±0,18	5,42±0,45
			48,7±11,0	41,8±0,9	33,4±0,5	17,9±0,8	9,80±0,13	9,8±0,16	5,14±0,36
		P	>0,05	>0,05	>0,05	0,045	<0,001	<0,001	>0,05
35	11th-12th days of pregnancy	IV	48,4±1,0	42,1±0,9	34,4±0,9	15,0±0,9	11,5±0,18	12,4±0,23	5,24±0,05
			48,1±1,0	40,2±0,9	33,2±0,8	18,4±0,7	9,00±0,01	9,8±0,17	5,16±0,01
		P	>0,05	>0,05	>0,05	<0,01	<0,001	<0,001	>0,05
35	13th day of pregnancy	V	46,0±0,7	37,8±0,7	32,4±0,6	16,7±0,5	9,35±0,10	11,2±0,17	5,10±0,03
			47,7±0,9	41,7±0,9	33,1±0,9	17,6±0,8	8,97±0,12	9,8±0,17	5,20±0,05
		P	>0,05	<0,001	>0,05	>0,05	0,01	<0,001	>0,05
35	15th-16th days of pregnancy	VI	51,2±1,4	40,9±0,9	33,9±0,8	16,1±0,7	9,65±0,12	11,1±0,13	5,26±0,12
			49,7±1,1	43,7±1,5	33,2±0,8	17,4±0,7	8,9±0,11	8,9±0,11	5,22±0,03
		P	>0,05	>0,05	>0,05	>0,05	<0,001	<0,001	>0,05
35	17th-18th days of pregnancy	VII	50,0±0,9	43,7±1,2	33,9±0,9	15,6±0,7	10,2±0,13	10,7±0,15	5,23±0,47
			50,5±1,1	43,0±0,9	32,4±0,8	17,0±0,8	8,9±0,13	9,4±0,16	5,21±0,42
		P	>0,05	>0,05	>0,05	>0,05	<0,001	<0,001	>0,05
Intact mice		VIII	48,2±1,2	41,6±0,9	33,4±0,9	18,4±0,8	8,7±0,16	9,8±0,16	5,18±0,03

Legend. Figures above show experiment, figures below show control.

EXPERIMENTAL RESULTS

In all 19 MMIT, migration of macrophages obtained from donor mice with nephrosonephritis was inhibited. The migration index averaged 0.13 ± 0.01. No inhibition of migration of macrophages obtained from healthy donors was found in the presence of kidney antigen in any of 20 tests. The migration index was over 0.8. The difference between the experimental and control results was statistically significant ($P < 0.001$). The results indicate that lymphocytes sensitized to kidney antigen are present in mice with nephrosonephritis.

Injection of lymphocytes from healthy syngeneic donors at different times of pregnancy (10th, 11th-13th, 15th-19th days) and before conception had no significant effect on the state of their young. With respect to total body weight and relative weights of the organs (kidneys, liver, lungs, heart) these newborn mice did not differ statistically significantly from intact ones. Injection of lymphocytes from donors with nephrosonephritis in a dose of 15 million cells also had no statistically significant effect on the body weight of the newborn mice or the relative weight of their organs, whereas injection of lymphocytes from affected donors in a dose of 35 million at different times of pregnancy (10th, 11th-13th, 15th-19th days) caused a statistically significant ($P < 0.05$) decrease in the weight of the newborn mice in all series of experiments (1.135 ± 0.03, 1.183 ± 0.02, 1.162 ± 0.02 g) compared with the weight of the control mice (1.285 ± 0.04, 1.206 ± 0.03, 1.262 ± 0.04 g), whose mothers received an injection of lymphocytes from healthy donors at the same time of pregnancy.

In the experimental newborn mice whose mothers received lymphocytes from donors with nephrosonephritis before the beginning of pregnancy in a dose of 35 million cells, not only a decrease in total body weight, but also a statistically significant ($P < 0.05$) increase in the relative weight of the kidneys was observed (expressed as a percentage of total body weight 0.60 ± 0.01% in the experiment and 0.55 ± 0.01% in the intact control), and also an increase ($P = 0.003$) in the weight of the lungs (2.1 ± 0.07% in the experiment and 1.8 ± 0.01% in the intact control), and an increase ($P < 0.01$) in the weight of the liver (5.8 ± 0.4%) compared both with intact control mice (4.3 ± 0.2%) and newborn young mice (4.2 ± 0.04%), whose mothers had received injections of lymphocytes from healthy donors.

These results are evidence that lymphocytes sensitized to kidney antigen, if injected into recipient mice

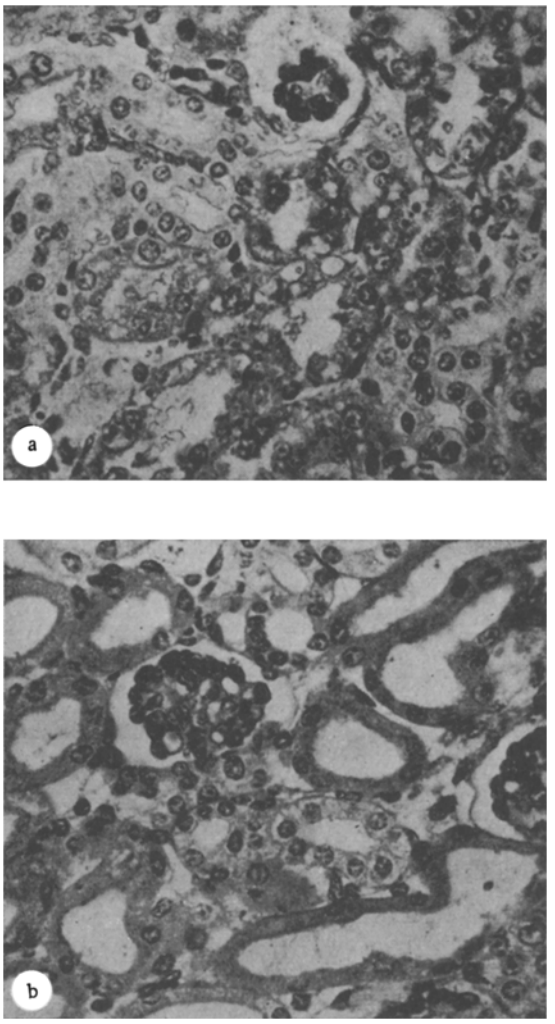

Fig. 1. Kidneys of newborn mice born to mothers receiving (on 11th day of pregnancy) lymphocytes taken from syngeneic donors with nephrosonephritis (a) and healthy donors (b). a) granular detritus in lumen of capsule of renal corpuscle, and cloudy swelling of cells of proximal portions of nephrons, with marked karyolysis; b) renal corpuscles and proximal parts of nephron of control newborn mouse. Stained with Schiff's reagent, 500×.

in a dose of 35 million cells, especially before pregnancy, had a toxic effect on the fetus, which was reflected in the body weight of the newborn animal and the relative weight of its organs.

It will be clear from Table 1 that the dimensions of the renal corpuscles and glomeruli in the experimental newborn mice of nearly all series except V did not differ from their dimensions in the control. Meanwhile, in all the experimental mice a statistically significant increase was observed in the size (height and width) of the cells of the convoluted tubules, the highest cells being found in the experimental mice of series II-IV. Correspondingly, in the experimental newborn mice of series II-IV the lumen of the convoluted tubules was statistically significantly narrowed compared with the control. These data show that the increase in size of the cells took place predominantly on account of their apical part. It will also be clear from Table 1 that when lymphocytes sensitized to kidney antigen were injected in a dose of 35 million, the dimensions of the cells of the convoluted tubules increased more than when these cells were injected in a dose of 15 million.

In the experimental newborn mice of series I and II, compared with the controls, the diameter of the cell nuclei was increased statistically significantly.

Histological analysis of the kidneys in the experimental newborn mice of all series revealed marked degeneration of the cells of the tubules, together with considerable karyolysis. The number of lysed nuclei in the experimental newborn mice of series IV was 36%, and of series V, 30%, whereas in the corresponding control newborn mice they numbered only 13%, and in the intact newborn mice only 6%. Thickening of the basement membrane was observed in the renal corpuscles with deposits of granular material in the lumen of the capsule (Fig. 1).

Lymphocytes of syngeneic donors, sensitized to kidney antigen, if injected into female animals at different times of pregnancy and before conception, besides their nonspecific toxic effect, induced changes in the kidneys of the newborn mice (an increase in size of the cells of the convoluted tubules, marked karyolysis). The early stages of embryogenesis, including the period of laying down of the metanephros (10th-12th days), proved to be most vulnerable. However, further investigations are necessary to establish the concrete mechanism of this effect of lymphocyte sensitized to kidney antigen on the fate of the kidneys in the progeny.

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ROLE OF COATED VESICLES IN SYNAPTOGENESIS IN MAN

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Despite the ever-increasing number of investigations of the phenomenon, synaptogenesis still remains a largely enigmatic process. This is particularly true of ideas on the mechanisms of the rapid differentiation of postsynaptic membranes, the structural and functional organization of which largely determines the specific features of the maturing synapse.

The complexity of the postsynaptic membrane, which includes a complex of special receptor and enzyme proteins, indicates quite definitely that its growth and differentiation must take place under strict genetic control. However, where and how these membranes are created and mature, and how they or their individual components reach the site of the future synapse also are still unsolved problems, and this naturally gives rise to many hypotheses and conjectures.

The most probable of these suggestions is the hypothesis of the exclusive role of the so-called coated vesicles in the genesis of the postsynaptic membrane and, perhaps, of the whole subsynaptic complex of the nerve cell [1-3], although of course there are other equally probable alternatives to this hypothesis [4, 5].

The object of this investigation was to study the possible role of coated vesicles in processes of synaptogenesis during the period of prenatal development of the nervous system in man.

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