Influence of High Level of Antibodies to Myelin Basic Protein in Female Mice on the Postnatal Development and Behavioral Reactions of the Progeny

S. G. Morozov, I. E. Gribova, T. P. Klushnik*, A. A. Sidyakin, B. B. Gnedenko, I. Yu. Toropygin, D. A. Fadeev, S. A. Zozulya*, E. I. Kovernaya, and Z. V. Sarmanova*

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Physical development, behavioral reactions, and training capacity were studied in the progeny of female BALB/c mice with high levels of antibodies to myelin basic protein. The proposed protocol of immunization ensures high levels of antibodies to myelin basic protein in this mouse strain. High level of antibodies to myelin basic protein in pregnant females causes an increase in the blood level of these antibodies in the progeny. Inhibitory effect of antibodies to myelin basic protein on physical development, training process, and memory in mouse pups was detected.

Key Words: *myelin basic protein; immunization; antibodies; behavioral reactions of mice*

Changes in the humoral immunity of women during pregnancy can lead to unfavorable aftereffects for the progeny. It was shown on experimental models that high level of antibodies (AB) to nerve growth factor in the blood of pregnant females inhibited physical development and impair learning of the progeny [2]. Increased levels of autoAB to neuroantigens, *e.g.* protein S100, myelin basic protein (MBP), anion nonhistone chromatin proteins (ACBP-C) and membrane proteins (MP-C) fraction, are associated with high incidence of pathologies (including nervous diseases) in newborns and infants aged 1-1.5 years [1,4]. However, it is still unknown, changes in the levels of which, specifically, AB lead to this or that disease.

It is known that the MBP family is involved in the regulation of the earliest events of brain development [7] and, presumably, with the regulation of other aspects of early development depending on MBP capacity to modify the status of the cytoskeleton components and of its catabolism products to stimulate the mitotic activity of cells.

We tried to detect in experiments probable relationship between high levels of AB to MBP in pregnant mouse females and their progeny and studied the effects of these AB on the development of newborn animals and their behavioral reactions.

MATERIALS AND METHODS

The study was carried out on BALB/c mice (21±2 g) from Kryukovo Breeding Center.

After adaptation, group 1 animals (n=9) were immunized with MBP, then three times with 10-day intervals with $F(ab)_2$ fragments of antiidiotypical antibodies (AAB) to MBP and after 1 month a single repeated dose of MBP was administered (10 μg antigen in 300 μl saline in complete Freund's adjuvant, 1:1 ratio, per animal).

N. E. Bauman Municipal Clinical Hospital No. 29, Health Care Department; Research Center of Mental Health, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** biopharm@list.ru. S. G. Morozov; igri@land.ru. I. E. Gribova

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Group 2 mice (n=5) were immunized with $F(ab)_2$ fragments of rabbit nonimmune IgG (10 μg in saline with complete Freund's adjuvant per animal) and group 3 animals (controls; n=4) were injected with saline with complete Freund's adjuvant 3 times with 10-day intervals and repeatedly after 1 month. Immunization was carried out subcutaneously in the axillary and inguinal area and along the abdominal white line.

MBP was isolated from cattle brain as described previously [6]. Antiidiotypical antibodies to MBP were obtained from hyperimmune sera of rabbits immunized with MBP by immunoaffinity chromatography; in order to isolate AAB, the sera were passed through a column with first-order AB immobilized on CH-sepharose, and in order to obtain AB, through a column with MBP immobilized on CH sepharose. Nonimmune IgG were obtained from rabbit sera by ammonium sulfate precipitation. Immunoglobulin $F(ab)_2$ fragments were obtained by pepsin cleavage.

Blood specimens were collected from mice after immunization and AB were measured by EIA; after high titers were detected, the females were mated with males. Progeny was obtained: 20 newborns from 2 females in group 1, 27 from 4 females in group 2, and 10 in the control group. Nursing females with the newborns were placed into individual cages and kept at 21-23°C and 12:12 day:night regimen (day from 7.00 till 19.00) with free access to water and fodder.

Four weeks after birth the pups were weighed, serum AB to MBP, $F(ab)_2$ fragments of AAB to MBP, and $F(ab)_2$ fragments of nonimmune IgG were measured. Unconditioned reflexes (grasping, turning, paw support test) were tested; total motor activity of mouse pups, number of running and rearing episodes were estimated in the rat-o-matic test.

Behavioral reactions were studied in an elevated plus-maze elevated 50 cm above the floor and consisting of 2 open arms and 2 arms with walls (25×5×20 cm) with a central square (5×5 cm). The animal was placed into the center of the maze with its head to the open arm; a piece of dry fodder was placed in the far end of this arm; the animal was to find it and memorize its location. The experiment was carried out for 6 min; the time needed to find the fodder was recorded. The experiment was carried out with each animal twice daily until manifestation of significant differences between the groups (6 days). The training capacity was evaluated by the time needed to find the fodder; anxiety was evaluated by the number of excursions into the open arms and time spent there. During the experiment the animals received fodder once daily (in the evening) and had free access to water. The capacity to memorize the acquired habit was evaluated by a single repeated testing in the maze 21 days after the end of the experiment.

Motor coordination and muscle force of the hind limbs was evaluated by the duration of stay on a rod: the animal was placed on a smooth wooden rod 2 cm in diameter, 30 cm elevated above the floor. The fore paw muscle strenght was evaluated by the time of holding the wire: the hind paws were fixed and the animal was allowed to grasp with its fore paws and hold the wire, stretched horizontally at the height of 30 cm above the table.

Serum AB levels were measured by EIA and expressed in optical density units.

The data were statistically processed using Student's t test.

RESULTS

The BALB/c mice were chosen for the experiment because injection of MBP to these animals does not lead to the development of experimental autoimmune encephalitis. The mice were immunized with MBP by alternating the native antigen (MBP) and $F(ab)_2$ fragments of AAB to MBP (immunochemical analogs of antigen), as it was impossible to obtain satisfactory titers of antiMBP in this mouse strain by the usual immunization protocols (MBP alone), because this mouse strain is genetically tolerant to this protein [5,8]. After immunization according to this protocol the production of AB to MBP is paralleled by production of AB to immunoglobulin $F(ab)_2$ fragments, and therefore we have added a group of mice immunized with $F(ab)_2$ fragments of nonimmune rabbit IgG.

The levels of antiMBP in group 1 mice were significantly higher than in groups 2 and 3, in which the titers were at the baseline levels (Table 1). On the other hand, higher levels of AB to $F(ab)_2$ fragments of AAB to MBP in group 1 (p<0.001) and to $F(ab)_2$ fragments of nonimmune IgG (p<0.001) in comparison with antiMBP were presumably due to different immunogenic activity of these antigens for mice. Significantly higher levels of AB to $F(ab)_2$ fragments of AAB to MBP in group 1 in comparison with group 2 indicate that in group 1 AB reacting with this antigen are directed mainly against specific MBP-like antigenic determinants. On the other hand, the levels of AB to MBP in mice of this group correlate with the levels of AB to $F(ab)_2$ fragments of AAB to MBP (r=0.82; p<0.01).

The levels of serum antiMBP in group 1 progeny were significantly (p<0.001) higher than the levels of AB in the progeny of groups 2 and 3 and

TABLE 1. Level of AB in Pregnant Females (*M*±*m*)

Group	Level of AB, opt. dens. units		
	to MBP	to $F(ab)_2$ fragments of AAB to MBP	to $F(ab)_2$ fragments of nonimmune rabbit IgG
1	0.213±0.054*	0.406±0.086*++	0.672±0.053*+
2	0.064±0.019	0.124±0.023	0.578±0.052
3	0.062±0.003	0.063±0.002	0.064±0.004

Note. *p<0.001 compared to group 3; +p<0.01, ++p<0.001 compared to group 2.

were 0.510±0.209 optical density units. Comparative analysis of the mean levels of antiMBP in group 1 progeny showed significant differences between animals (0.694±0.112 and 0.327±0.072 optical density units, respectively; p<0.001), AB levels being higher in the progeny of mothers with higher levels of AB. The level of AB to $F(ab)_2$ fragments of nonimmune IgG in the progeny of group 2 was 0.616±0.088 optical density units, which was 1.5 times higher than in group 1. The level of AB to MBP and $F(ab)_2$ fragments of nonimmune IgG in control pups was no higher than the baseline (0.1 optical density units). Hence, high level of AB in the blood of pregnant females was associated with similar elevation in the progeny, the levels of antiMBP in the progeny correlating with those in pregnant females.

The duration of pregnancy in group 1 mice was 5 days shorter than normally. The mean weight of pups in this group at the age of 1 month was significantly lower than in the control (Table 2). An opposite effect was observed in the progeny of pregnant females with AB to $F(ab)_2$ fragments of nonimmune IgG. The weights of all mice of this group at the age of 1 month was significantly (p<0.000001) higher than in group 1 and control.

Two mice born from mothers with higher levels of antiMBP had pareses of the hind limbs (one mouse later died), half of mice from both mothers had tremor of the hind paws. These symptoms were compensated by the age of 1 month. Testing of muscular strenght showed that the duration of stay on the rod and holding the wire in this group was significantly (p<0.004 and p<0.00026, respectively) shorter than in the control, the duration of stay on the rod being the less, the lower were antiMBP levels (r=-0.38). No differences between the control group and mice with high levels of AB to $F(ab)_2$ fragments of nonimmune IgG were detected in these tests. Analysis of correlations between the time of holding the wire and stay on the rod showed a high correlation between these values in the control group (r=0.85; p<0.01), indicating comparable strength of the fore and hind limbs in these animals and hence, their proportional physical development. The correlation detected in group 2 mice (r=0.55) was insignificant because of little number of observations, while in group 1 no correlation of this kind was detected.

On the whole, the results indicate that the development of group 1 mice was delayed, the degree of this delay correlated with the levels of antiMBP. Hence, high level of AB to MBP in the blood of animals during stages of embryonic and early postnatal development modified the course of gestation in females and significantly inhibited physical development of the progeny.

Testing of unconditioned reflexes (grasping, turning, paw support attempts) showed no significant differences between the groups.

A trend to prolongation of the time needed to find the food in the maze was noted in group 1 mice on day 6 (1.5 times longer than in the control; Fig. 1). Group 2 mice needed the longest time to take the food, the differences from group 1 and controls were significant (p=0.0003 and p=0.0001, respectively). However, this fact can be explained not only by lower learning capacity in this group, but also

TABLE 2. Physical Development of Mouse Pups (*M*±*m*)

Group	Weight, g	Time of stay on the rod, sec	Time of holding the wire, sec
1	22.3±4.1*	37.9±47.2**	2.18±1.37**
2	33.6±3.2**	102.4±73.9	8.16±5.70
3	25.01±7.84	103.0±71.0	7.11±4.70

Note. *p=0.05, **p<0.001 compared to group 3.

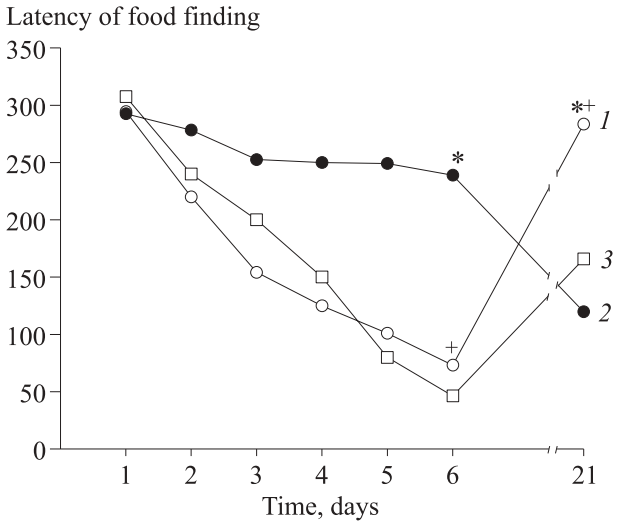

Fig. 1. Latency of food finding by mice of different groups in an elevated plus-maze. *1*) group 1; *2*) group 2; *3*) group 3. **p*<0.001 compared to group 3; +*p*<0.001 compared to group 2.

by higher resistance to food deprivation of these animals due to higher body weight.

The retention of acquired habit was tested after 21 days in an elevated plus-maze. Group 1 mice exhibited the longest latency of finding the food, their value differing significantly from group 2 (p=0.00006) and 3 (p=0.004). In group 1 animals this parameter on day 21 was the same as on day 1 of testing. The differences between groups 2 and 3 on day 21 were negligible; the time of finding the food in group 3 corresponded to that on days 3-4. These data indicate virtually complete loss of acquired habit in group 1 mice and its partial retention in control group. Our data on the loss of acquired habit in little mice with high level of antiMBP are in line with previous data on the effect of high level of AB to nerve growth factor on the progeny [2], which suggests, that high level of AB to various neuroantigens in the blood of animals during embryonic and early postnatal development can result in reduced training capacity.

Statistical processing of the data on the number of entries into open arms of the maze and time spent there reflecting anxiety of animals [3] revealed no differences between the groups, which suggests that antibodies had no effect on mental stress reactions.

No differences in the number of running episodes and motor activity of little mice were detected in a common, non-stressogenic situation (rat-o-matic test), though the number of rearing postures was significantly higher in group 2 animals (p<0.001) than in groups 1 and 3.

The findings indicate a relationship between physical and neurological deviations in mice with high level of antiMBP in their mothers, which can indirectly reflect some mechanisms of malformation of the nervous system in children born from mothers with high levels of antiMBP [1]. An important experimental factor is that changes in the humoral immunity, not linked with increased level of AB to neuroantigens, are also essential for physical development and learning processes in the progeny.

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