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Social stress in laboratory rats *Rattus norvegicus* results in decreased immune competence of the offspring

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An immune system status of socially stressed laboratory rats *Rattus norvegicus* Berkenhout, 1769 and their offsprings was studied. Several tests on activity of the macrophages and lymphocytes as well as on the tumour necrosis factor a (TNFa) activity in the blood serum were used to assess an immune system status of the stressed and control groups of rats. The results indicated that social stress led to obvious alterations in the immune system of the stressed rats and especially of their offspring, where the changes were more pronounced.

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Introduction

Consequences of the high density and social stress impact have been demonstrated in both natural populations studies and laboratory experiments (Tompson 1957, Christian and Davis 1964, Shilov 1977, Moshkin *et al.* 1990, Naumenko *et al.* 1990, Pratt and Lisk 1990, Zakharov *et al.* 1991). Changes in an organism's condition has been indicated by developmental stability decrease revealed for the progeny of socially stressed pregnant females of rat (Valetsky *et al.* 1997). However, it is also important to answer the question if these morphological alterations are accompanied by changes in an immune status, as one of the crucial parameters of an organism's condition and its viability.

To detect possible changes not only in stressed individuals, but also in their progeny, is essential to estimate the population consequence of the impact. The latter seems to be especially important, as it has been shown that viral infections

at the pregnancy duration lead to more serious changes in immune status of the offsprings than in the infected mothers (Pronin *et al.* 1989, Kobets *et al.* 1995).

High lability of the immune system suppose a convenience of immunological tests to register any stress impact, including the social one. One of the early manifestation of stress is a discharge of granulocytes from the storages, a redistribution of lymphocytes followed by depletion of thymus and spleen (Ernström and Sandberg 1973, Cohen and Crnic 1985). Activity of the cells provided a natural resistance (natural killer cells, mononuclear cells) is also decreased under the stress influence (Plezity *et al.* 1987, Aguila *et al.* 1988). All these alterations finalised in a rise of the susceptibility to oncologic and infectious diseases (Lewis *et al.* 1983, Francois *et al.* 1987, Gotovceva *et al.* 1988). However, the data concerning the stress influence on immune competent cells proved to be not so definitive. Stress can be followed by activation of lymphoid cells (Sukhikh *et al.* 1983, Korneva and Schkhinek 1989), depression of neoplastic cell growth (De Chambre and Gosse 1973, Rabin and Salvin 1987), etc. The consequences of stress depend upon various parameters, including genetically controlled sensitivity to the stressor, an immune system status (Laudenslager *et al.* 1983, Lysle *et al.* 1989), etc. Activity of different cell subsets and different functions of a particular cell subset can be changed discordantly (Lysle *et al.* 1988). Therefore the crucial task is to make a right choice of the immunological tests allowing to predict the consequences of stress for the lymphoid organs and cells. Several tests on activity of the macrophages and lymphocytes as well as on the tumour necrosis factor a (TNFa) activity in the blood serum have been established as rather simple and effective system to assess the immune system status (Pronin *et al.* 1993, 1996).

The aim of the study was to test the selected immune parameters in rats exposed to the social stress as well as in their offsprings to detect possible changes in immune status under the stress impact.

Material and methods

Laboratory rats *Rattus norvegicus* Berkenhout, 1769 ("Bright") derived from the R. Trayon's strain "maze-Bright", selected according to their extrapolation ability revealed in the test with the complex labyrinth, were used in the study. Social stress was modelled by the maintenance of 2 pregnant females with 8 males in one cage. Each female in a control group was kept in a separate cage during 22 days of pregnancy (see Valetsky *et al.* 1997 and Borisov *et al.* 1997 for details of the experiment and strain description). Ten females from the control and 10 from the experimental groups (all 6 months old) were used for immunological study. Fourteen offsprings from the control group and 15 offsprings from the experimental group (all 1.5 months old) were tested.

The following parameters were included in the study to characterize the immune system status:
(1) Bone marrow and thymus cell numbers were estimated according to the procedure described earlier (Pronin *et al.* 1996).
(2) Activity of different phagocyte enzymes, protein synthesis, tumour necrosis factor a (TNFa) production and phagocytosis were tested to assess the natural resistance (Zaytseva *et al.* 1988).
(3) Phagocytic activity of peritoneal macrophages was examined by ingestion of 14C-labeled *Salmonella typhi vaccine*. The level of protein synthesis was estimated by uptake of 14C-labeled aminoacids through

the standard procedure (Zaytseva *et al.* 1988). Results of these two tests were expressed as a count per minute (cpm) for 1 ml of the sample or for 1 mg of the protein, respectively.
(4) Cathepsin D activity was determined by the Anson's test (Anson 1936). The activity of cathepsin D in macrophages and serum was expressed as a quantity of tyrosine (mg) produced by cathepsin D during an hour per 1 mg of the protein.
(5) For determination of 5'-nucleotidase the non-organic phosphorus was measured in macrophages of the peritoneal exudate by the multiscan MCC at 630 nm wave length. The level of enzymatic activity for the control animals was considered to be 100% (Kirillicheva *et al.* 1988, Pronin *et al.* 1996).
(6) To estimate the level of the TNFa in serum we used the L929 target cells following the standard procedure (Le and Vilcek 1987, Pronin *et al.* 1996). Conventional units of activity were calculated by the serum dilution caused 50% lysis of the target cells.
(7) T- and B-cell proliferation induced by mitogens was estimated by 3H-thymidine uptake into the DNA of cells incubated with mitogens. The radioactivity was measured at a scintillation b-spectrometer. The results were expressed as the cpm values (Pronin *et al.* 1989, 1996).

The arithmetical mean and standard error (SE) were calculated for each test under study, and *t*-statistics was used for the intersample comparison of the means (Sokal and Rohlf 1981).

Results

Some indications for an increase in number of bone marrow cells and a decrease in a thymus weight have been revealed under stress for both females and their progeny, that were more pronounced for the latter (Table 1). Among the parameters presented in Table 2 there is a slight tendency for a decrease in a cathepsin D activity in peritoneal macrophages under stress impact for females. In offsprings of stressed females the same tendency proved to be much more pronounced ($p < 0.001$). There was also significant decrease of phagocytosis in stressed offsprings as compared with the control ones ($p < 0.05$).

The tendency for a decrease in activities of 5'-nucleotidase and NaK-ATPase in stressed females, comparing with the control group, became highly significant for their progeny ($p < 0.001$) (Table 3). Stress impact did not lead to an appearance of TNFa in the serum of females. TNFa in offsprings of the control rats was found in significant titres in 4 animals, while in offsprings of the stressed rats it was

Table 1. Bone marrow cellularity and thymus weight (mean ± SE) in different experimental groups of rats. * significant ($p < 0.05$) difference between control and stressed group revealed by *t*-test.

Groups	*n*	Cellularity of bone marrow (10^6/femur)	Thymus weight (mg)
		Mothers	
Control	3	70.0 ± 24.7	192.7 ± 18.7
Stressed	3	71.1 ± 8.7	181.7 ± 9.3
		Offsprings	
Control	3	33.8 ± 8.9	175.3 ± 26.6
Stressed	3	41.7 ± 12.4	104.3 ± 19.5*

Table 2. Functional activity of phagocytes (mean ± SE) in different groups of rats. * $p < 0.05$, *** $p < 0.001$ (t-test).

Groups	n	Phagocytosis	Protein synthesis	Cathepsin D in: peritoneal macrophages	Cathepsin D in: blood cells	Cathepsin D in: serum
			Adult			
Control	10	10.1 ± 0.5	167.1 ± 22.8	415.0 ± 37.1	198.0 ± 25.6	18.0 ± 3.3
Stressed	8	11.1 ± 1.6	256.4 ± 67.9	337.0 ± 22.7	230.0 ± 11.6	15.7 ± 1.9
			Offspring			
Control	14	37.8 ± 6.5	243.6 ± 36.1	571.0 ± 37.1	153.0 ± 17.8	11.2 ± 1.6
Stressed	15	19.1 ± 2.0*	239.0 ± 38.6	348.0 ± 37.4***	170.0 ± 19.1	13.0 ± 2.7

Table 3. Determination of 5'-nucleotidase and NaK-ATPase activities (mean ± SE) in peritoneal macrophages in different groups of rats. *** $p < 0.001$ (t-test).

Groups	n	5'-nucleotidase activity (%)	NaK-ATPase activity (%)
		Adult	
Control	7	100.0 ± 8.2	100.0 ± 6.7
Stressed	10	96.7 ± 2.2	89.5 ± 4.0
		Offspring	
Control	7	100.0 ± 8.2	100.0 ± 6.7
Stressed	7	38.9 ± 3.2***	50.3 ± 2.9***

Table 4. Proliferative activity of spleen cells (mean ± SE) in different groups of rats. * $p < 0.05$, *** $p < 0.001$ (t-test)

Groups	n	^{3}H-thymidine uptake (cpm) in cell cultures: without mitogens	with Con A	with LPS
		Adult		
Control	5	2336 ± 310	25875 ± 925	2308 ± 155
Stressed	4	1346 ± 110*	13737 ± 186***	1286 ± 59***
		Offspring		
Control	5	1689 ± 34	11721 ± 249	2204 ± 40
Stressed	5	1300 ± 60***	8962 ± 234***	1167 ± 33***

found only in 1 animal (the mean values of titres in conventional units for the control and experimental groups proved to be equal to 60.6 ± 9.3 and 10.0 ± 10.0 respectively, $p < 0.01$).

The same tendency for a decrease in the spontaneous and induced lymphocyte proliferation can be seen in both stressed females and their offsprings as compared with the control groups (Table 4). The difference between the experimental and control groups proved to be statistically significant for all parameters studied.

Discussion

An increase in bone marrow cellularity and a decrease in thymus weight appeared to be a compensatory reaction directed to the enrichment of an immune competent cells pool. This increase of bone marrow cells number can be a result of their migration from thymus followed by its hypoplasia. The latter can be also a consequence of the lymphocyte mortality (Zimin 1983, Gordeyeva and Nikolaeva 1991). Some indications of the quantitative changes in an immune system characterized by an increase in a cellularity of bone marrow and hypoplasia of thymus more noticeable in the offsprings of stressed rats (Table 1) could be a manifestation of these processes.

Different results indicating the stimulatory and inhibitory effects of stress impact on macrophage functional activity have been observed in the earlier studies (Loose *et al.* 1984, Plezity *et al.* 1987). In our study the social stress followed by alterations in some parameters of the macrophage activity. The most noticeable changes were observed in the offsprings whose macrophage phagocytic activity and cathepsin D activity were sharply decreased. The analysis of TNFa activity confirmed this conclusion.

Disappearance of TNFa in offspring of stressed rats can be a result of the macrophage function depression. An adenisine, as the 5'-nucleotidase product, is considered to be as one of the main physiological regulators of the immunological functions (Kirillicheva *et al.* 1988). Stimulation of the immune system is commonly accompanied by a decrease of the 5'-nucleotidase activity. We revealed a significant decrease in the activity of this enzyme for the offsprings of the stressed rats only (Table 3).

T-cells proliferation induced by mitogens has been established as the most informative test for evaluation of stress (Broom and Johnson 1993). Various stress impact is commonly followed by a proliferation decrease (Monjan and Collector 1977, Hoffman-Goetz *et al.* 1986, Odio *et al.* 1986, Rabin and Salvin 1987, Cunnick *et al.* 1988). In our study the indications for a decrease of T-lymphocyte proliferation with ConA in the stressed animals and their progeny were found (Table 4). These findings proved to be in agreement with the data on the negative correlation of macrophage and T-cell activities (Biozzi *et al.* 1984).

It has been mentioned that T-cells could be more sensitive to stress than B cells (Korneva and Schkhinek 1989). However, there are some data showing that a noise can lead to a decrease of B-cell proliferation with LPS (Monjan and

Collector 1977). It has been found that various stress impact could lead to a suppression of B-cell activity and to a decrease in number of the antibody forming cells (Gisler 1974, Boranic *et al.* 1984, Esterling and Rabin 1987, Keller *et al.* 1988, Kirillina *et al.* 1989). Our data also showed a tendency to decrease of B-cell activity in the stressed rats (Table 4).

Thus, several immunological parameters under study indicate a depression of the immune system under the stress impact as compared with the control group. The changes revealed prove to be much more pronounced in the offsprings than in the females stressed at the pregnancy duration. This result corresponds to the earlier suggestion that stress impact at the pregnancy duration could have more serious consequences on the immune status and viability of the progeny than of the stressed females (Pronin *et al.* 1989, Kobets *et al.* 1995). The results also support an importance of developmental stability decrease revealed for the progeny of socially stressed females (Valetsky *et al.* 1997) and demonstrate that stress impact could lead to serious changes in an organism's condition and population consequences, as it has been suggested in the earlier field studies (Zakharov *et al.* 1991).

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