

STRESS AND IMMUNITY-INFLAMMATION INTERACTIONS.

II. INFLUENCE OF EXERCISE UPON THE HUMORAL IMMUNE RESPONSE IN RATS CHALLENGED WITH AN INFLAMMATORY AGENT

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Summary

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A rat model of inflammation, provoked by oyster glycogen, was used to evaluate the influence of exercise-induced stress upon the immune response against sheep red blood cells (SRBC). The applied stress was accompanied by reduced B-lymphocyte counts and decreased serum antibody titers – both total and 2-mercaptoethanol (2ME)-reduced haemagglutinins and haemolysins (IgG), that increased less significantly compared to controls and did not reach control peak values. The antibody-forming cells (AFC) counts were also lower compared to controls between days 7 and 8.

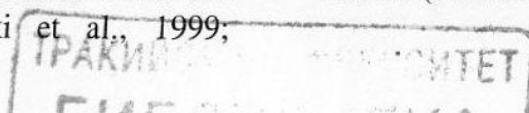
Following the application of the non-bacterial inflammation agent oyster glycogen to SRBC-immunized rats (control group), an increase in B-lymphocyte counts and in serum total and 2ME-reduced haemagglutinin and haemolysin titers was observed. The AFC counts in spleen were slightly reduced at day 8.

Key words: inflammation, humoral immune response, stress

INTRODUCTION

The influence of various stressors and inflammatory agents upon the organism provokes a complex of adaptive reactions, mediated by the nervous and immune systems. The activation of hypothalamic-pituitary-adrenal axis (HPAA) and the increment of stress hormones in blood are responses to those effects and in the inflamed area, both synthesis and release of multiple mediators of inflammation occur. Those biologically active substances, being included in regulatory pathways and counterpart interactions, induce important inflammatory and immunological changes (Hoffman-Goetz and Pedersen, 1994; Suzuki et al., 1999;

Vuitton et al., 1999). In conditions of stress, those changes are most commonly related to glucocorticoid (GC)-mediated suppression of immune system and/or sympathetic systemic-modulated mechanisms (Sternberg and Licinio, 1995; Webster et al., 1997). In previous studies of ours, a suppression of cellular and humoral immune responses was observed in both acute and chronic swimming stress with a degree of suppression, correlating with the duration of exercise. It was more pronounced in chronic stress, where a much higher corticosterone level was observed (Gundasheva, 1993).



The stress with increased GCs during inflammation was found to initiate multiple changes in the cytokine spectrum (Webster, 1998) and the production of mediators of inflammation (Buckingham and Flomer, 1997), to influence the synthesis and traffic of leukocytes resulting in lymphocytopenia, monocytopenia, eosinopenia and neutrophilia (Chrousos and Gold, 1992; Chrousos et al., 1993; Gundasheva, 1997) and to exert antiinflammatory effects.

The aim of the present study was to follow out the combined effect of a continuous and strenuous physical exercise and experimental inflammation with oyster glycogen upon some parameters of the humoral immune response in rats.

MATERIALS AND METHODS

Animals

The experiment was performed on male inbred Wistar rats weighing 140–160 g at the age of 2.5 months. Two weeks prior to the study, the animals were divided into 2 groups: control group (n=18), that included rats with inflammation and experimental group (n=18) – with stress and inflammation. Rats from both groups were kept in cages in groups of nine under a 12 h daylight/12 h dark regimen and an ambient temperature of 20–22 °C. They had a free access to commercial granulated feed and water. All experiments were performed between 8 a.m. and 12 a.m. to exclude the influence of circadian cycles.

Experimental stress

Stress was provoked by swimming with a burden (3% of body weight) at 20 °C to full exhaustion for five consecutive days at the same hour of the day. This stress model was previously described and had been shown to be associated with the highest corticosterone elevation (Gundasheva, 1993).

Immunization

For primary immune response, 1 ml suspension of $10^9/l$ sheep red blood cells (SRBC) in saline was injected intraperitoneally in both groups (immediately after the first seance of stress in experimental rats).

Experimental inflammation

It was provoked with a non-bacterial inflammatory agent – 5 ml of 0.9% NaCl, containing oyster glycogen (BDH, England) 8 mg/100 ml, injected intraperitoneally to controls and to experimental animals on the 5th day after the SRBC immunization immediately before the last seance of stress in experimental rats.

Immune status parameters

B-lymphocytes were determined according to Kajdacvy-Balla et al. (1976), the antibody-forming cells (AFC) counts – according to Cunningham (1965) in a lymphocytic spleen suspension; the antibody titer in rat serum – using a micro-method as total haemagglutinins (HA_T) and total haemolysins (HL_T) (Graffar and James, 1973) and 2-mercaptoethanol-reduced haemagglutinins (2ME-HA) and haemolysins (2ME-HL) (IgG) (Jeludkov and Chernisheva, 1987); haemolytic activity of complement in blood serum – according to Stelzner and Stein (1971).

All immune parameters were followed out at days 5, 7 and 8 after SRBC application that corresponded to hours 6, 48 and 72 following the oyster glycogen challenge.

Statistical analysis

The results were expressed as mean $\pm$ SEM and submitted to a two-way analysis of variance (ANOVA). *Post hoc* comparisons of individual group means were carried out by the least significance difference test (LSD) at the $p < 0.05$ level.

RESULTS

The dynamics of changes in humoral immune response parameters is presented in Table 1. In controls with inflammation, B-lymphocyte counts increased continuously up to day 8 (72 hours following oyster glycogen challenge) while the AFC counts decreased gradually till day 8. The titers of serum HA_T, HL_T, 2ME-HA and 2ME-

HL were the highest at day 7 and afterwards decreased at day 8.

In animals with stress and inflammation (experimental group), B-lymphocytes tended also to increased similar to controls, but the counts at days 7 and 8 were lower than those of unstressed animals. The AFC counts decreased with a minimum at day 8 that was statistically different from the counts at day 5. The changes

Table 1. Changes in B-lymphocytes, antibody forming cells (AFC), total haemagglutinins (HA_T), total haemolysins (HL_T), 2-mercaptoethanol-reduced haemagglutinins (2ME -HA), 2-mercaptoethanol-reduced haemolysins (2ME-HL) and complement in control (n=18) and experimental (n=18) rats. Data are presented as mean ± SEM (in brackets)

Parameters	Periods		
	Day 5	Day 7	Day 8
Control group (inflammation)			
B-lymphocytes, 10 ⁴	920 (110)	1300 (110) ^a	1770 (100) ^c
AFC, 10 ⁶	242 (27)	160 (40)	141 (14) ^b
HA _T , log2	6.2 (0.5)	11.3 (1.2) ^b	8.3 (0.7) ^a
HL _T , log2	12.2 (2.7)	22.2 (1.7) ^b	15 (1.1)
2ME-HA, log2	0.49 (0.5)	2.49 (0.6) ^a	0.66 (0.3)
2ME-HL, log2	3.16 (0.7)	7.49 (0.6) ^c	2.99 (0.9)
Complement*, CH ₅₀	399 (22)	430 (35)	365 (13)
Experimental group (stress and inflammation)			
B-lymphocytes, 10 ⁴	990 (120)	1080 (140)	1160 (70) ³
AFC, 10 ⁶	300 (28)	115 (15) ^c	62 (6) ^{c3}
HA _T , log2	4.2 (0.3) ²	7 (0.4) ^{c2}	6.3 (0.3) ^{c1}
HL _T , log2	13.2 (1.3)	15 (1.2) ²	14 (0.7)
2ME-HA, log2	0 (0)	0.6 (0.3) ²	0.16 (0.16)
2ME-HL, log2	2.32 (0.6)	5.32 (0.6) ^{b1}	1.83 (0.9)
Complement*, CH ₅₀	387 (32)	426 (15)	410 (21)

* haemolytic activity

Level of significance: ^a p<0.05; ^b p<0.01; ^c p<0.001 vs day 5; ¹ p<0.05; ² p<0.01; ³ p<0.001 between groups at each interval.

in those two parameters were more expressed in the experimental group at day 8 being statistically significant compared to the respective change in controls.

The dynamics of HA_T, HL_T, 2ME-HA and 2ME-HL titers in experimental rats was similar although the values of those parameters were lower than that in controls during the whole period of the study. The differences vs controls were statistically significant at day 7.

The changes in complement haemolytic activity in both groups were characterized by a slight increase at day 7.

DISCUSSION

Our results about B-lymphocyte counts and serum total and 2ME-reduced haemagglutinins and haemolysins (IgG) in controls evidenced a stimulation of the humoral immune response. Probably, macrophages are involved in this stimulation. The increase in those cells in peritoneal exudate between hours 48-72 after the oyster glycogen challenge (days 7 and 8 following SRBC application) was observed in previous studies of ours (Gundasheva et al., 1997). According to Northoff and Berg (1991), Kumar and Das (1994), Horai et al. (1998), activated macrophages synthesize multiple mediators, whose effect is related to the formation and performance of inflammation and cellular and humoral immune responses during inflammation.

The decrease in IgM-AFC was not probably due to the decrease in B-lymphocytes, because those cells tended to increase during the experimental period. It was most probably related to the enhanced suppressor activity of Fc γ -RFC, reported in another study (Gundasheva, 2001). This did not influence the serum antibody titers that remained elevated probably because of the increased degree

of immunoproliferative processes, observed histologically in lymph nodes (Gundasheva, 1993).

In the experimental group of rats, the B-lymphocyte population was influenced by stress. Although changes were similar to those in controls, there were statistically significant differences in their level as well as in the levels of AFC, HA_T, 2ME-HA, HL_T, 2ME-HL compared to controls. Furthermore, our data showed that the serum antibody titers in stressed animals did not follow the dynamics of AFC change. In previous studies of ours (Gundasheva, 1993) on rats subjected to swimming stress, at day 7 following stress, the medullae of lymph nodes were pathohistologically shown to contain plasmatic cells with various degree of maturity. Those cells or antibodies produced by them migrate in blood and increase antibody titers despite the AFC inhibition in spleen, observed between days 7 and 8.

The suppression of the humoral immune response could be due to various factors. Exercise-induced stress is related to HHPA activation and the release of GCs and catecholamines. The high GC level results in reduced number of immunoglobulin-synthesizing cells, lower CD4⁺/CD8⁺ ratio and decreased IL-2 production (Del Rey et al., 1984; Ader and Kohen, 1993; Faith, 1999). This type of stress, accompanied by inflammation, decreases the active E-rosette forming cells counts and in a lesser extent - Fc γ -RFC counts (Gundasheva, 2001). Obviously, the changes in the cellular immune response could provoke a reduced antibody production.

The humoral immune response could be negatively influenced by catecholamines released following stress (Lyte et al., 1991; Pedersen, 1991; Dobbs et al.,

1993) as well as by the sympathetic nervous system (Okimura et al., 1986; Bellinger et al., 1992; Peristein et al., 1992) and corticotropine-releasing hormone (Irwin, 1993; Vuitton et al., 1999).

In stress, combined with inflammation, the release of multiple mediators of inflammation is probably important as well. It is established that after a strenuous physical stress, the increased production of prostaglandins from monocytes suppress the B-cellular population and decrease the antibody counts (Pedersen, 1991; Kumar and Das, 1994; Hoffman-Goetz and Pedersen, 1994; Gromihina and Kozlov, 1996).

Furthermore, in strenuous exercise, the production of IL-6 and IL-1 β , representing mediators released also in inflammation, is elevated (Viti et al., 1986; Northoff and Berg, 1991; Pedersen, 1991; Hoffman-Goetz and Pedersen, 1994). Those proinflammatory cytokines are considered to trigger the HPA axis (Klasing and Johnstone, 1991; Peristein et al., 1992; Sternberg and Lucinio, 1995) and HPA-axis-mediated synthesis of GCs that regulate the macrophageal and B-cellular activity (Harbuz and Lightman, 1992; Celada and Nathan, 1994; Faith, 1999) during the course of inflammatory and humoral immune responses.

In our opinion, the combined effect of physical stress and inflammation resulted in changes in neuro-endocrine regulation that inhibit the humoral immune response. Probably, the production of cytokines and mediators in those states act secondarily as mechanism of immune regulation. Additional, more extensive studies are however needed to elucidate the precise mechanisms that mediate the reciprocal effects among stress, inflammation and immunity.

CONCLUSIONS

The influence of the non-bacterial inflammatory agent oyster glycogen is performed via increase of B-lymphocytes and the titers of serum total and 2ME-reduced haemagglutinins and haemolysins (IgG) in SRBC-immunized rats. The AFC counts were slightly decreased.

The combined effect of continuous and strenuous swimming stress and oyster glycogen challenge provoked a suppression in B-lymphocyte counts and serum antibody titers [total and 2ME-reduced haemagglutinins and haemolysins (IgG)] that increased less intensively compared to controls and did not reach their peak values. The AFC counts were also lower compared to controls.

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