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Thymic response of C57BL/6 mice to three different stressors

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Abstract

PÉREZ-MERA, M.L., GUERRA-PESTONIT, B. & REY-MÉNDEZ, M. (1993). Thymic response of C57BL/6 mice to three different stressors. *Nova Acta Científica Compostelana (Bioloxía)*, **4**: 173-177

Mice C57BL/6 exposure to three different stressed conditions (immobilization, cold or forced swimming) shows a different thymic response with respect to control animals. Thus, thymus weight is not affected by forced swimming but decreases in immobilized animals and increases in mice stressed by cold, in spite that corticosterone levels are higher in all three cases than in controls. These stress-induced changes affect the thymus population of immature thymocytes while the number of mature ones remains constant.

Key words: Stress, thymus, adrenals, corticosterone, thymocytes, spleen.

Resumen

PÉREZ-MERA, M.L., GUERRA-PESTONIT, B. & REY-MÉNDEZ, M. (1993). Respuesta tímica de los ratones C57BL/6 a tres diferentes tipos de agentes estresantes. *Nova Acta Científica Compostelana (Bioloxía)*, **4**: 173-177

Los ratones C57BL/6 expuestos a tres diferentes condiciones de estrés (inmovilización, frío y natación forzada) presentan una respuesta tímica diferente comparada con animales control. Así, el peso del timo no es afectado por la natación forzada pero decrece en animales inmovilizados y aumenta en ratones estresados por frío, a pesar de los superiores niveles de corticosterona encontrados en los tres casos con respecto a los controles. Estos cambios inducidos por el estrés afectan a la población tímica inmadura, mientras que la madura no se ve alterada.

Palabras clave: Estrés, timo, corticosterona, adrenales, timocitos, bazo.

INTRODUCTION

Stress contributes to the development and course of a range of illnesses by inducing changes in the immune function (DANTZER, R. 1991). Although stress has usually been associated with detrimental effects, mainly those ones derived from suppression of the thymus-dependent immune system, immunoenhancement and inhibition of tumor growth can result under certain experimental conditions (MONJAN, 1981; RILEY, 1981; JUSTICE, 1985). Animals response to stress includes involution of the thymus and other lymphatic organs (SELYE, 1936, 1973), that can be attributed to the action of adrenocortical hormones released in response to stress

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(KIRSCHBAUM *et al.*, 1992; SANTISTEBAN & DOUGHERTY, 1954; DECATANZARO *et al.*, 1991). So, plasma levels of these hormones, as well as increase in the size of the adrenal glands, are often used as a measure of the stress response.

Immunosuppression caused by the stress-induced rise of glucocorticoids can be counteracted by the capability of thymic hormones (thymosins) to block the binding of the steroid to its receptor by converting steroid-sensitive lymphocytes (immature T cells) to steroid-resistant lymphocytes (mature T cells) (HALL *et al.*, 1985). This is consistent with the hypothesis that thymus acts as an antistress organ, although the response of thymocytes subpopulations to stress is unknown.

Another well-established stress effect is a decrease in body weight gain (KOHLER & KNOSPE, 1982; ARMARIO *et al.*, 1984; MORMEDE *et al.*, 1984; DECATANZARO & MACNIVEN, 1992) not related to decrease in food intake (GAMALLO *et al.*, 1986) or to over-stimulation of the hypothalamic-pituitary-adrenal axis with a low production of growth hormone (ALARIO *et al.*, 1987).

The aim of this work is to investigate whether three different stressors (immobilization, cold or forced swimming) have similar or antagonic effects on C57BL/6 mice and to study the response to stress of thymus T cells subpopulations. For this reason, the stress application is followed by measures of lymphatic organ weights, plasma levels of corticosterone and adrenal weights, number of mature and immature thymocytes and body weight gain.

METHODS

Animals

Male mice of the C57BL/6 strain (35±2 days old), weighing about 20g were housed in groups of four per cage in a temperature- and light-controlled room with free access to food and water. Lights were on for 12 h/day begining at 08.00h; temperature was 22±1°C. Mice were weighed at the start and at the end of the experimental period in order to assess their rate of weight gain.

Experimental procedures

Animals were divided into four groups: (IS) immobilization stress, mice were immobilized by taping their four limbs in supine position to a board for 90min a day; (CS) cold stress, mice were placed in a -20°C freezer for 10min a day; (SS) forced swimming stress, it was made in a water tank (50cm diameter and 60cm high, temperature 18°C) during 5min a day; and (CO) control, mice were left undisturbed in the animal house for 14 days. Controls a stressed animals were maintained in recomended conditions for these studies (RILEY, 1981). Stress was performed in all cases during 14 days between 10.00 and 12.00am. Immediately after the last stress application and ether anesthesia, two random animals of each group were killed at the same time by decapitation and the trunk blood collected and centrifuged (1500xg, 10min) at 4°C. Serum was stored at -20°C until the measure of corticosterone levels (ZENKER & BERNSTEIN, 1958) and adrenals, spleen and thymus were removed and weighed.

Cell preparation

Thymuses were minced in cold phosphate-buffered saline (PBS), and dispersed by pressing through a stainless-steel mesh to obtain a single cell suspension. Erytrocytes were removed by treatment with a lysing buffer (17mM Tris-HCl and 144mM NH4Cl, pH 7.2) at 0°C for 10min. Cells were washed twice in PBS containing 1% (w/v) bovine serum albumin (BSA; SIGMA, USA), and resuspended in the same buffer. The fractionation of thymocytes was carried out by the peanut agglutinin (PNA; SIGMA, USA) method described by REISNER & SHARON (1984). In all the experiments cell viability was determined by trypan blue exclusion and only cell suspensions with a viability of 90% or over were used.

Statistics

Each result is expressed as the mean values ± S.E.M. of eight mice sacrified in groups of two every two weeks. Statistical evaluation was performed by one-way analysis of variance. Comparisons between groups were made by

Student-Newman-Keuls (SNK) test and $p \leq 0.05$ was considered significant.

RESULTS

The analysis of variance of experimental data shows significant differences among groups for all the studied parameters (data not shown). The analysis of the results for each parameter (summarized in Table I), indicates that immobilization stress was the most affected group, since their values are different in every case ($p \leq 0.01$) from the other groups (control, cold, swimming) except for the weight of the adrenal glands which does not differ from the swimming group.

Swimming stress seems to be the least affected group towards the studied parameters showing differences in body weight ($p \leq 0.05$) and in plasma corticosterone levels ($p \leq 0.01$) in comparison to control.

Cold stress affects every parameter except for the adrenal glands. In comparison to other groups, cold stress has significant differences in both thymus and spleen weight and in corticosterone levels, but while immobilization stress diminishes the size of thymus and spleen, cold stress, surprisingly, induces an increase of both lymphoid organs

Stress-induced changes on thymocytes total number correlate with the thymus weight (Fig. 1): immobilization stress presents the smallest cell number (showing a 64.34% higher than control) and cold stress increases the cell number (48.00% higher than control). Swimming stress is the least affected (22.51% smaller than control).

Cell separation by PNA shows cellular variations affecting the PNA^+ cells whereas PNA^- cells show no significant differences (from 11.72×10^6 in immobilization to 12.27×10^6 in control).

DISCUSSION

Our results indicate that all the studied parameters are affected by the three different stressors although with different signs and intensity depending on the stressor used. Thus, we found that immobilization stress is the only one that correlates their responses with the effects pointed out by SELYE (1936, 1973): thymic and spleen involution; body weight decreasse; increase in both adrenal weight and plasma levels of corticosteroids. Physiological consequences of such stress mediated events have significant adverse effects on important elements of the immunological apparatus that can be translated in pathological effects of a stress-associated

TABLE I. Body weight gain, gland weights and corticosterone levels (mean±sem) for control and stressed C57BL/6 mice (n=8/group)

Parameter Group	Body[a] (g)	Thymus (mg)	Spleen (mg)	Adrenals (mg)	Corticosterone (µg/dl)
CO	8.51±0.16	74.84±2.14	91.84±5.12	5.07±0.41	4.50±0.29
IS	2.79±0.12 $p \leq 0.01$[b]	42.50±1.47 $p \leq 0.01$	57.35±2.43 $p \leq 0.01$	6.64±0.31 $p \leq 0.01$	38.42±2.96 $p \leq 0.01$
CS	7.93±0.31 $p \leq 0.05$	94.20±1.93 $p \leq 0.01$	100.59±1.60 $p \leq 0.05$	5.40±0.17 N.S.[c]	18.55±2.04 $p \leq 0.01$
SS	7.70±0.17 $p \leq 0.05$	70.79±5,21 N.S.	86.07±1.83 N.S.	5.73±0.40 N.S.	25.42±0.97 $p \leq 0.01$

Groups: CO= control, IS= immobilization stress, CS= cold stress, SS= forced swimming stress.

[a] Body weight gain was determined as an increase of body weight in the experimental period (14 days).
[b] Compared with control.
[c] No significant compared with control ($p > 0.05$).

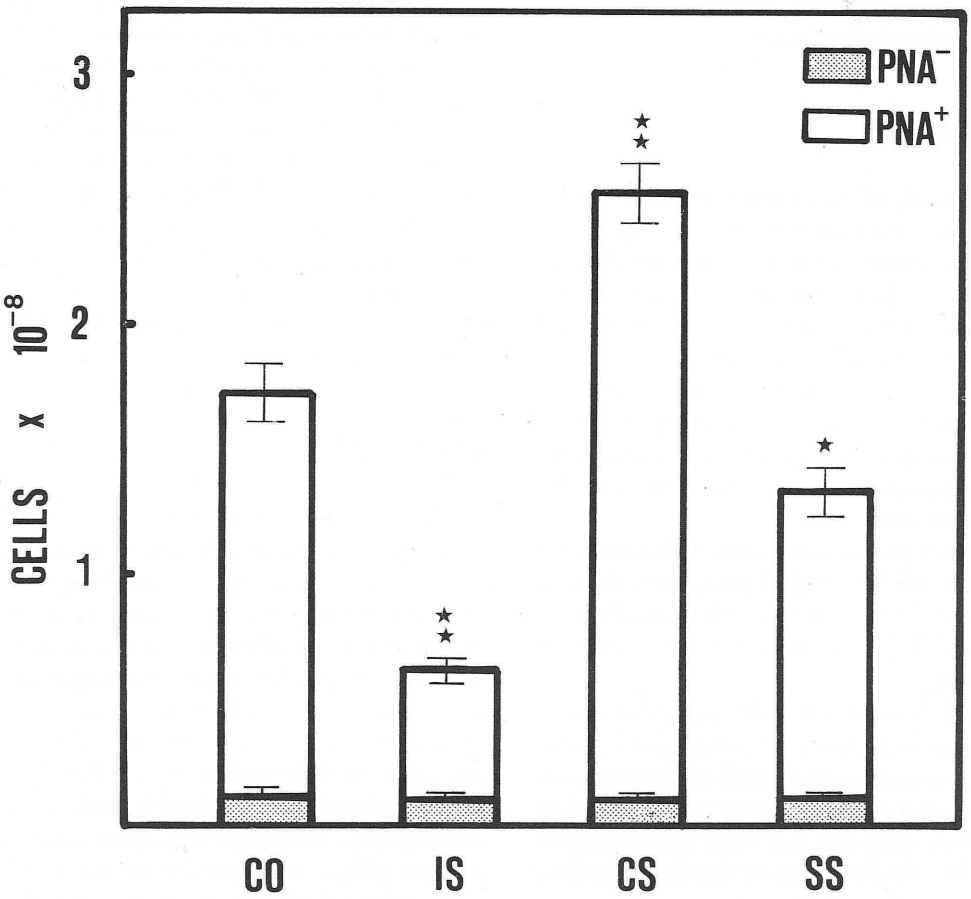

Fig.1. Effect of stressors on PNA fractionated thymocytes. Columns indicate the cell number of PNA^+ and PNA^- thymocytes from: control (CO), immobilization stress (IS), cold stress (CS) and forced swimming stress (SS) animals. Values are means±S.E.M. (n=8). Significant differences from control of PNA^+ cells by SNK test: $^*p\leq0.05$, $^{**}p\leq0.01$. No significant differences were found on PNA^- cells.

decrease in immune competence on cancer processes, virus infection and other diseases subject to immunological control (RILEY, 1981; DANTZER, 1991).

Forced swimming is the stressor with the less influence on the studied parameters and the most significant difference respect to control is the level of plasma corticosterone. This may be due to two factors: one of them is the kind of stressor used, that depending on its control or not by the individual could influence on metabolic parameters (KEVETNANSKY *et al.*, 1987). The other one would be the duration of stress because the intensity of the response is related to the time and intensity of the stress exposure (KELLER *et al.*, 1981).

Cold stress, on the other hand, does not show a typical stress response since it induces a weight increases of the studied lymphoid glands. This response does not seem to correlate the corticosterone increase with the thymic involution and can be explained, the rise of corticosterone, as a neurogenic or emotional response to a novel and unpleasant environment rather than a response to the cold itself (GIBBS, 1985).

We found that the changes in thymus weight induced by the three different stressors are related to an increase or decrease in the number of immature thymocytes (PNA^+) while the number of mature ones (PNA^-) remains constant. These results are contrary to the mechanisms (HALL *et al.*, 1985) that postulates an increase in the number

of mature cells to compensate the immunosuppressor effects of corticosteroids.

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