Stress and immunity-inflammation interactions. I. Changes in some parameters of immune response under the influence of stress in rats, challenged with an inflammatory agent

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SUMMARY

The aim of the study was to follow out the changes in active and total E-rosette forming cells (Ea-RFC, Et-RFC) and Fcγ-rosette forming cells (Fcγ-RFC) under the simultaneous influence of strenuous exercise stress (swimming) in rats immunized with sheep red blood cells (SRBC), challenged by the non-bacterial agent oyster glycogen.

SRBC-immunized rats without inflammation (group A) showed increased Ea-RFC, Et-RFC and Fcγ-RFC counts with a peak at day 7. In immunized rats with inflammation (group B), this increase was more significant compared to group A, with a peak at day 8. The stress, associated with inflammation in SRBC-rats (group C) resulted in significant suppression of Ea-RFC and Et-RFC counts at day 8 (p < 0.001) and influenced negatively, but less significantly Fcγ-RFC counts at day 7 (p < 0.05) evidencing a suppressed immune response.

The application of stress without inflammation (group D) decreased Ea-RFC and Fcγ-RFC counts in the early phase of immune response (day 5) as well as Et-RFC counts, especially at day 8 (p < 0.001). This matched the lower Et-RFC counts in rats with stress and inflammation, suggesting the immunosuppressive effect of applied stress.

KEY-WORDS : stress - inflammation - immunity - rats.

RÉSUMÉ
Stress et relations immuno-inflammatoires. I. Changements de certains paramètres de la réponse immunitaire sous l’influence du stress chez des rats atteints d’une inflammation. Par D. GOUNDASHEVA. Département de Pathologie Générale et Clinique, Faculté de Médecine Vétérinaire, Université de Thrace, 6000 Stara Zagora, Bulgaria

Le but de l’étude est de déterminer les changements dans les cellules actives et totales formant des E-rosettes (Ea-RFC et Et-RFC) et les cellules formant Fcγ rosettes survenus à la suite de l’action combinée d’une fatigue physique exténuante (par la nage) et d’une inflammation provoquée par un agent non-bactérien glycérogène d’huître chez des rats immunisés aux érythrocytes ovins (EO).

Le nombre des Ea-RFC, Et-RFC et Fcγ-RFC chez les rats sans inflammation (immunisés aux EO), (le groupe A ) est plus élevé et a un maximum le 7ème jour. Chez les rats immunisés aux EO et ayant une inflammation (groupe B) ces indices sont beaucoup plus élevés par rapport au groupe A et le maximum est le 8ème jour. Chez les rats immunisés aux EO (groupe C), le stress associé à une inflammation, mène à une suppression marquée du nombre de l’Ea-RFC et de l’Et-RFC le 8ème jour (p < 0.001) et influence négativement mais faiblement Fcγ-RFC le 7ème jour (p < 0.05), ce qui démontre une suppression de la réponse immunitaire des cellules.

La seule application du stress sans inflammation (groupe D) diminue le nombre de Ea-RFC et du Fcγ-RFC dans la phase initiale de la réponse immunitaire (5ème jour) et réduit sensiblement le nombre de Et-RFC le 8ème jour (p < 0.001). Cela coïncide avec la réduction du nombre de Et-RFC observée chez les rats stressés et enflammés en faisant penser au rôle immuno-suppressif du stress appliqué.


Introduction

The activation of the hypothalamic-pituitary-adrenal axis (HPAA) during stress is one of the primary pathways for regulation of the extent of immune and inflammatory responses from the part of the central nervous system [4, 18]. There is evidence that stress response is integrated via the nervous and immune systems and that there is a bidirectional activity between them. Recently these interactions have been related to the involvement of cytokines, released by the activated lymphocytes and other cells during stress as well as during inflammation [3, 20]. Thus, the HPAA is triggered in order to prevent their overproduction and therefore, their significant catabolic effect and also to preserve the systemic homeostasis. The final stage of its activation is related to the incretion of glucocorticoids, exercising multifunctional and deep effects upon the control of cell-mediated immunity and
inflammation, forming the classical feedback control [1, 5, 13]. Previous studies of ours have shown that exercise-induced stress alters the lymphocyte functions in rats [6]. Moreover, exercise stress influences adversely the course of inflammation suppressing fever, affecting the cytokine production of lymphocytes and macrophages and modifying blood cellular composition [7, 26].

The mechanisms of the simultaneous effect of exercise-induced stress and inflammation upon the immune response are scarcely studied. That is why the aim of the present study was to follow out the influence of stress achieved by strenuous exercise upon some parameters of the cellular immunity in rats challenged by the non-bacterial agent oyster glycogen.

Material and methods

1) ANIMALS

The experiments were performed on male inbred Wistar rats weighing 150 ± 10 g, aged 2.5 months. The animals were divided into 4 groups: group A (n = 18) - rats without inflammation; group B (n = 18), that included rats with inflammation, group C (n = 18) - with stress and inflammation and group D (n = 18) - with stress. All rats were fed and housed uniformly.

2) STRESS

The stress consisted in 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 procedure has been shown to be associated with a high corticosteroid elevation [6].

3) IMMUNIZATION

For primary immune response, the rats from all groups were injected intraperitoneally with 10⁶ sheep red blood cells (SRBC) in 1 ml of saline. For groups C and D, the immunization was performed after the first swimming procedure (Fig. 1).

4) 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 the animals from groups B and C on day 5 following the SRBC immunization. For the latter group, the inflammatory agent was applied before the last stress challenge.

5) DETERMINATION OF EA-RFC, ET-RFC AND FCγ-RFC

The determination of active E-rosette forming cells (Ea-RFC), total E-rosette forming cells (Et-RFC) and Fcγ-rosette forming cells (Fcγ-RFC) was performed by the method of rosette formation in a spleen lymphocyte suspension. The method is not expensive, easy to perform and sensitive for studying immunological reactions. It was used for a quantitative characteristics of antigen-specific lymphocytes, forming rosettes with SRBC.

a) Preparation of lymphocyte suspension

Tissues from spleens were collected immediately after cervical dislocation of the rats and were placed in PBS cooled on ice. After a careful removal of adjacent tissues, they were cut to small pieces (2-3 mm) and the cells were cautiously released by teflon homogenizer. The cellular suspension was filtered through a fine stainless steel screen in the presence of medium 199 (Sigma Chemicals Co., St. Louis, USA). The lymphocytes were separated by gradient centrifugation in Lymphoprep™ (Nycomed AS, Oslo, Norway), washed twice in Hank’s solution. The subsequent processing of lymphocytes was done according to immune methods, described below.

b) Determination of total E-RFC

The total E-RFC were prepared according to the method described by BIOZZI [2]. The lymphocyte suspension, obtained after separation (see 5.a.), was washed twice with medium 199 (Sigma Chemical Co., St. Louis, USA) by centrifugation (200 x g ; 10 min ; 4 °C). Lymphocytes were resuspended to a concentration of 2 x 10⁷ cells/ml. Their viability, assayed with 0.1 % trypan blue (Merck, München, Germany) was not less than 95 %.

SRBC used for the test were obtained from the blood of one ram, sampled aseptically with an anticoagulant (1 ml 10 % sodium citrate per 9 ml blood). After a triple washing with buffer, SBC were adjusted to 2 x 10⁸ cells/ml.

The cellular sediment, obtained after mixing of aliquots of adapted lymphocytes and erythrocytes, was centrifuged (200 x g ; 10 min ; 4 °C). The supernatant was removed. The pellet was carefully resuspended in 1 ml medium 199 and thereafter incubated at 37 °C for 1 h.

Rosette counting was done with a light microscope in a Bürker chamber (Transmdimpex, Vienna, Austria) by counting 100 medium squares. A lymphocyte, binding three or more SRBC was accepted as rosette. The results were presented as Et-RFC counts (x 10⁴).

c) Determination of active E-RFC

Active E-RFC were determined according to the method of SMITH et al. [23]. The achievement of normal Ea-RFC counts requires the use of a proper ration between SRBC and lymphocytes (40 : 1) ensuring a maximum cell interaction and thus, there is no more a need for preliminary incubation of lymphocytes for 1 h and the use of fetal calf serum. Such rosettes are formed only by lymphocytes, possessing receptors with a high affinity towards SRBC.

Briefly, aliquots of 2 x 10⁶ cells/ml lymphocytes and 0.5 % SRBC suspension were mixed and centrifuged immediately (200 x g ; 10 min ; 4 °C). The supernatant was removed. The pellet was carefully resuspended in 1 ml medium 199 and the counting - done as described in 5.b.

d) Determination of Fcγ-RFC

The identification of Fcγ-RFC with suppressor activity was done by the rosette test of GUPTA and GOOD [8] as followed:

a) the isolation of the lymphocytes from spleen was done as described in 5.a.

b) the purification of the T-lymphocytes was carried out by means of rosette-formation with SRBC because in SRBC-immunized rats, T-lymphocytes possess receptors for these erythrocytes. After the formation of the rosettes, the SRBC were lysed with buffered 0.83 % ammonium chloride, the separated T-lymphocytes were resuspended in a RPMI 1640 medium (Sigma Chemical Co, St. Louis, USA) containing inactivated foetal calf serum and penicillin, streptomycin and were incubated overnight at 37 °C. At the end of the incubation period, the pellet was washed 3 times in Hanks and was resuspended to a concentration of 4 x10⁶ cells/ml.

c) the preparation of purified rabbit anti-Ox IgG antibodies was done in the National Center for Infectious and Parasitic Diseases, Sofia, Bulgaria.

d) the preparation of Ox-RBC-antibody complexes was done by incubating an equal volume of 2 % Ox-RBC and anti-Ox-RBC IgG antibody (1 : 100 dilution) at room temperature for 24 hours. The complexes were washed three times in Hanks and resuspended to a concentration of 1 %.

e) the Fcγ-RFC preparation consisted in mixing 100 µl T-lymphocyte suspension with 100 µl Ox-RBC-antibody complexes and centrifugation (150 x g, 5 min, 4 °C). The supernatant was removed and the pellet - resuspended in 1 ml medium 199, followed by incubation at 37 °C for 1 hour. The counting was done as described in 5.b.

The immune response was followed out at days 5, 7 and 8 after SRBC application corresponding to hours 6, 48 and 72 after the oyster glycogen challenge respectively.

6) STATISTICAL ANALYSIS

The results were expressed as mean ± SEM and submitted to a statistical analysis of variance (ANOVA). Post-hoc comparisons of individual group means were carried out by the least significant difference test (LSD).

Results

The changes in Ea-RFC, Et-RFC and Fcγ-RFC counts are presented in Table I. In group A (without inflammation), there was an increase in those cell counts with a maximum at day 7 after the SRBC application.
In rats from group B (with inflammation), a similar tendency was present but the peak occurred at day 8. The average Ea-RFC, Et-RFC and Fcγ-RFC counts were higher during the whole experimental period compared to group A. The combined effect of inflammation and stress (group C) resulted in increased Ea-RFC and Et-RFC counts at day 7 (48 h following oyster glycogen administration) and a subsequent decrease by day 8. Compared to group B at the same intervals, Ea-RFC and Et-RFC counts were however lower by day 7 and particularly by day 8 (p < 0.001). Fcγ-RFC counts in that group were lower vs group B at day 7 (p < 0.05). Ea-RFC and Et-RFC at day 5 were significantly higher compared to rats without inflammation (group A). The application of stress without inflammation in SRBC-rats (group D) was accompanied by lower Ea-RFC and Fcγ-RFC at day 5. Afterwards, Ea-RFC counts increased by day 7 (p < 0.001) and although lower by day 8 (p < 0.001), remained significantly higher vs day 5. A similar tendency towards elevation was observed for Fcγ-RFC, especially at day 8 (p < 0.01 vs day 5). Et-RFC counts were however gradually decreasing reaching a minimum by day 8 (p < 0.001 vs day 5). Ea-RFC counts in this group were lower at day 5 (p < 0.05) compared to rats without inflammation (group A) and day 8 (p<0.01) compared to rats with inflammation (group B), but were higher at day 7 (p < 0.05) compared to group C. A statistically significantly lower Et-RFC counts vs group A and B were observed during the whole period of the study, whereas vs group C - at days 7 and 8. There were no significant differences in Fcγ-RFC counts in group D compared to groups A, B or C.

**Discussion**

Our data indicate that the immunization of rats from group A (without inflammation) stimulated a normal immune response, manifested by a gradual increase in Ea-RFC, Et-RFC and Fcγ-RFC counts with a peak at day 7. However, in rats with inflammation (group B), those counts were much more elevated, the tendency towards increase - similar and the peak is shifted up to day 8 after SRBC immunization (i.e. at hour 72 following oyster glycogen challenge). Thus, we conclude that the used model of inflammation possibly stimulated the cellular immune response.

Earlier [7] we observed that in rats challenged with oyster glycogen, the counts of lymphocytes and non-lymphoid leukocytes (neutrophils, basophils, eosinophils, monocytes and macrophages) in the peritoneal exudate were elevated. Most probably, the white blood cells, activated in the inflamed area, release multiple mediators that are important for the initiation and maintenance of immune response and influence the course of inflammation.

In group C (stress and inflammation) we observed an impaired immune response in the T-system expressed in decreased Ea-RFC, Et-RFC and Fcγ-RFC counts compared
to group B. This decrease could possibly be influenced by the lymphopenia (because of the enhanced migration of lymphocytes in the bone marrow), observed earlier as well as by the dystrophic and lytic processes in the thymus, spleen and lymph nodes at the background of the high corticosterone levels using the same model of stress [6]. An easy lymphocyte lysis in steroid-sensitive species like rats following a glucocorticoid challenge is observed by others too [5].

The application of stress without inflammation (group D) resulted in decreased Ea-RFC and Fcy-RFC counts in the early phase of immune response (day 5) and decreased significantly Et-RFC counts during the productive phase of immune response at day 8 (p < 0.01) that matched the Et-RFC decrease in group C. This allows us to suggest that the applied model of stress could possibly promote the inhibition of cellular immune response in rats with both stress and inflammation.

The inhibition of suppressive activity of T-lymphocytes and the active rosette formation following stress in the inductive phase of immune response is reported by other authors [11, 12, 13] inhibit the production, activation and secretion of monokines like IL-1, TNFα, IL-2, that regulate not only the immune and inflammatory responses as cortyco- tropin-releasing hormone (CRH) whose immunosuppressive effects are direct [19], HPAA-mediated and sympathetic system-mediated [10, 22]. There are more and more data about the influence of IL-1, released from activated macrophages which, acting centrally via HPAA stimulation towards release of CRH, ACTH and glucocorticoids during stress, could cause a cellular immunity suppression [16, 24, 25].

It is possible that the decrease in Ea-RFC, Et-RFC and Fcy-RFC in experimental rats from group C was due to the influence of other hormonal factors as adrenaline that inhibits CD4+ T-cells following stress [15, 21] as well as to cortico- tropin-releasing hormone (CRH) whose immunosuppressive effects are direct [19], HPAA-mediated and sympathetic system-mediated [10, 22].

The detailed elucidation of the mechanism of stress upon the course of the inflammatory and immune responses requires additional studies, mainly upon the interactions among stress hormones and the cytokines IL-1, IL-6, TNFα and IL-2, that regulate not only the immune and inflammatory responses but influence the neuroendocrine system as well.

Conclusions

The effect of the non-bacterial agent oyster glycogen in SRBC-immunized rats, was manifested by increase in Ea-RFC, Et-RFC and Fcy-RFC.

The combined effect of continuous strenuous exercise-induced stress (swimming) and oyster glycogen-induced inflammation had a strong inhibiting effect on Ea-RFC, Et-RFC and in a lesser extent, on Fcy-RFC in SRBC-immunized rats.

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References


