Brain, Behavior, and Immunity 26 (2012) 50–60

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Chronic cold stress in mice induces a regulatory phenotype in macrophages: Correlation with increased 11β-hydroxysteroid dehydrogenase expression

R. Sesti-Costa *, M.D.C. Ignacchiti, S. Chedraoui-Silva, L.F. Marchi, B. Mantovani

Department of Biochemistry and Immunology, Ribeirão Preto Medical School, University of São Paulo, 14049-900 Ribeirão Preto, SP, Brazil

ARTICLE INFO

Article history:
Received 4 April 2011
Received in revised form 13 July 2011
Accepted 14 July 2011
Available online 22 July 2011

Keywords:
Neuroimmunomodulation
Chronic stress
Macrophage
Phagocytosis
11β-HSD
Glucocorticoid
Catecholamine
Growth hormone

ABSTRACT

Susceptibility to infections, autoimmune disorders and tumor progression is strongly influenced by the activity of the endocrine and nervous systems in response to a stressful stimulus. When the adaptive system is switched on and off efficiently, the body is able to recover from the stress imposed. However, when the system is activated repeatedly or the activity is sustained, as during chronic or excessive stress, an allostatic load is generated, which can lead to disease over long periods of time. We investigated the effects of chronic cold stress in BALB/c mice (4 °C/4 h daily for 7 days) on functions of macrophages. We found that chronic cold stress induced a regulatory phenotype in macrophages, characterized by diminished phagocytic ability, decreased TNF-α and IL-6 and increased IL-10 production. In addition, resting macrophages from mice exposed to cold stress stimulated spleen cells to produce regulatory cytokines, and an immunosuppressive state that impaired stressed mice to control *Trypanosoma cruzi* proliferation. These regulatory effects correlated with an increase in macrophage expression of 11β-hydroxysteroid dehydrogenase, an enzyme that converts inactive glucocorticoid into its active form. As stress is a common aspect of modern life and plays a role in the etiology of many diseases, the results of this study are important for improving knowledge regarding the neuro–immune–endocrine interactions that occur during stress and to highlight the role of macrophages in the immunosuppression induced by chronic stress.

1. Introduction

Stress can be defined as a state of homeostatic disturbance induced by psychological, environmental, physiological or infectious stimuli. Neuroendocrine changes are triggered as an adaptive reaction to stress stimulation, and the changes induced greatly differ according to type, intensity and duration of stress. Physiological responses to stress were observed by Selye in 1949 and referred to as "General Adaptation Syndrome" (Selye and Fortier, 1949). Currently, this adaptation to a stressor is referred to as allostasis, which is an essential, active and adaptive process for maintaining a steady state via multiple effectors (McEwen, 1998). Stress responses are mediated by two main mechanisms: (a) activation of the hypothalamic–pituitary–adrenal (HPA) axis, which culminates in glucocorticoid secretion, and (b) stimulation of the sympathetic nervous system (SNS), which results in the release of norepinephrine and epinephrine (Laurentiis et al., 2005; Sternberg, 2006; Tausk et al., 2008; Webster et al., 2002).

When this adaptive system is switched on and off efficiently, the body is able to recover from the stress imposed. However, when the system is activated repeatedly or the activity is sustained, an allostatic load is generated, which can lead to disease over long periods of time (McEwen, 1998). During a stress response, the neuroendocrine system helps the body to cope with the stressor by preparing the body for a "fight or flight" reaction, which can enhance or inhibit some aspects of the immune system (Dhabhar, 2002; Glaser et al., 2000). The final result depends on intensity of stressor stimulus, its continuity or intermittence as well as the immune cells evaluated. A large part of the immune response that is important for resolving infectious, tumoral and inflammatory injuries requires the involvement of macrophages, essential cells that efficiently remove cell debris generated by apoptosis during tissue remodeling or necrosis resulting from trauma. Macrophages are also responsible for the recognition and destruction of foreign agents in the body through phagocytosis and the production of reactive oxygen species. Finally, macrophages are fundamental for driving the T cell response through antigen presentation and cytokine secretion (Mantovani et al., 2007; Mosser and Edwards, 2008).

Studies on the effects of stress on macrophages are contradictory. Some reports have indicated a decreased phagocytic ability upon stress induction (Garbulinski et al., 1991; Palermo-Neto et al., 2003), whereas others have shown an increase in phagocytosis in response to stress (Barriga et al., 2001; Ferrandez and De la Fuente,

* Corresponding author. Fax: +55 16 3633 6840.
E-mail address: renata.sesti@usp.br (R. Sesti-Costa).

0889-1591/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2011.07.234

1999; Shilov and Orlova, 2003). This divergence in results may be explained by differences in the type and duration of the stress applied in the study, the activation state of macrophages and particularly, the receptors involved in phagocytosis, as different studies use different types of particles for macrophage ingestion. Additionally, it is not known whether macrophages under stress are able to influence the T cell response.

Our previous studies have shown that acute cold stress model acts differently on resting and lipopolysacharide (LPS)-activated macrophages. The acute stress model consisted of a single submission of mice to 4 °C for 4 h, which was able to increase plasma concentrations of corticosterone, epinephrine and norepinephrine. Resting macrophages from acutely stressed mice exhibited lower phagocytosis mediated by Fcγ and mannose receptors, whereas activated macrophages had higher phagocytic capacity by the same receptors. Besides, the latter were less able to phagocytosis of apoptotic cells at the inflammatory site and to produce TGF-β (Baccan et al., 2010; Sesti-Costa et al., 2010). In the current study, we aim to investigate the effect of chronic stress, submitting mice to 4 °C for 4 h during seven consecutive days, on the function of resting and LPS-activated macrophages under various stimulation conditions and determine whether macrophages from stressed mice could influence the T cell response.

2. Methods

2.1. Animals and chronic cold stress

Male 6- to 8-week-old BALB/c mice were obtained from Ribeirão Preto Medical School, University of São Paulo animal center and maintained under a 12:12 light:dark cycle with food and water available *ad libitum*.

Chronic stress was induced by exposing mice to 4 °C for 4 h each day for 1, 6, 7 or 14 days, and the mice were sacrificed immediately after last session of stress. The protocol of stress used was approved by the ethics committee of the Faculty of Medicine of Ribeirão-USP (protocol No. 134/2006).

2.2. Quantification of plasma hormones

After exposure to stress, mice were decapitated, and blood was collected into heparin-coated tubes. Blood was centrifuged, and the plasma was stored at -70 °C until use in assays. For catecholamine measure, plasma was stored in sodium metabisulfite to avoid oxidation. Catecholamines were purified by adsorption in alumin at pH 8.8 and dosed by HPLC using an octadecylsilane column (ODS-C18) and electrochemical detection as previously described (Garofalo et al., 1996).

For corticosterone dosage, steroids were extracted from the plasma by adding 1 ml of ethanol. Corticosterone concentrations were determined by radioimmunoassay as previously reported (Vecsei, 1979) using an rabbit anti-corticosterone antibody and ^{3}H-corticosterone as the competitor. The radioimmunoassay performed for growth hormone (GH) was standardized according to Szepeshazi and co-workers (Szepeshazi et al., 2001) using a rabbit anti-rat GH antibody and a recombinant mouse GH (National Hormone & Peptide Program (NHPP), CA, USA). Mouse GH was labeled with 0.6 mCi I^{125} by Chloramine T, and the labeled hormone was purified on a Sephadex G75 column. Plasma samples (100 μl) from mice were incubated with the anti-rat GH antibody and 10,000 cpm of GH-I^{125} at 4 °C for 72 h. The secondary antibody was added, and samples were incubated at 4 °C for 24 h. Polyethylene glycol was then added, and samples were centrifuged to precipitate the immune complexes. The supernatant was discarded, and the reading was performed on the precipitate.

2.3. Isolation of peritoneal macrophages

Immediately after exposure to stress, animals were sacrificed by cervical dislocation, and macrophages were harvested by injecting 3 ml of Hanks' Balanced Salt Solution (HBSS) into the peritoneal cavity. The cell suspension was incubated on culture plates for 60 min at 37 °C in RPMI-1640 (Sigma) containing 10% fetal bovine serum (Life Technologies, New York, NY, USA) for the macrophages to adhere as described by Mantovani (Mantovani, 1987) and washed with HBSS to remove the non-adherent cells.

For experiments with activated macrophages, mice were intraperitoneally injected with 50 μg of LPS (lipopolysaccharide from *Escherichia coli* 026: B6 – Sigma, St. Louis, MO, USA) in 500 μl of PBS four days before the collection of macrophages. After this period, cells from the peritoneal cavity consisted of more than 95% macrophages, as seen by optical microscopy.

2.4. Phagocytosis assay

Immune complexes were produced by staining goat red blood cells (GRBCs) with PKH26 (Sigma) according to the manufacturer's instructions and incubating them for 30 min at 37 °C with mouse anti-GRBC antibody, which was produced and purified as previously described (Mantovani, 1987). Apoptotic thymocytes were obtained by incubation with 1 μM dexamethasone for 3 h at 37 °C, and they were then stained with cell tracker green CMFDA (Molecular Probes, Invitrogen, Eugene OR, USA) according to the manufacturer's instructions. Zymosan was resuspended in carbonate buffer with 25 μg/ml FITC (Sigma) for 30 min at 37 °C and incubated with mouse serum to promote opsonization.

Macrophages (10^6) were incubated at 37 °C for 45 min with 500 μl RPMI-1640 (Sigma) medium containing 10% fetal bovine serum and the different phagocytic stimuli, including an immune complex of IgG bound to red blood cell-PKH26 (4×10^6), zymosan-FITC (50 μg), zymosan-FITC opsonized with complement (50 μg) and apoptotic thymocyte-CMFDA (5×10^6).

The macrophages incubated with apoptotic thymocytes were washed vigorously using a Pasteur pipette to remove the surface bound stimuli as described by Licht and co-workers (Licht et al., 1999), and red blood cells from the immune complex bound to macrophages were lysed by hypotonic shock as described by Mantovani (Mantovani, 1987). The fluorescence of internalized particles was captured by flow cytometry (FACSCanto, BD Biosciences) after fluorescence quenching of zymosan-FITC particles bound to the macrophage surface with trypan blue (2 μg/ml). Results were analyzed using FlowJo® (Tree Star) software and represented as the mean fluorescence intensity (MFI) per macrophage.

2.5. Superoxide anion determination

Peritoneal cells were suspended in Hanks' containing 1% gelatin (Difco, Detroit, MI, USA) to prevent the adhesion of macrophages to the tubes, and 10^6 cells were incubated with 0.15 mM lucigenina (Sigma) for 5 min at 37 °C. The immune complex of IgG bound to OVA was prepared by incubation of 1 mg/ml OVA for 1 h at 37 °C with antibody anti-OVA, which was prepared and purified as previously described (Lucisano and Mantovani, 1984). Zymosan or zymosan opsonized with complement was prepared as item 2.4. The stimuli were added to a final concentration of 200 μg/ml. The luminescence generated by the reaction was captured immediately for 90 min, and the results were expressed as the peak of superoxide release.

2.6. Actin polymerization

Macrophages (5×10^5) were adhered onto coverslips, fixed and permeabilized with 2% paraformaldehyde containing 0.3% Triton