ACUTE LIPOPOLYSACCHARIDE ADMINISTRATION IMPAIRED IMUNE RESPONSIVENESS IN NORMAL RATS

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Abstract: Involving of endotoxin lipopolysaccharide (LPS) on immune response modulation was examined in adult male Wistar rats. Acute LPS injection (25µg/kg, i.p, Sigma) increased serum corticosterone, suggesting significant effects on hypothalamic-pituitary-adrenal axis (HPAA) activity. In addition, acute LPS administration significantly decreased the number of leukocyte, the number of lymphocyte, total serum protein, antibody titer, body weight and significantly increased albumin-globulin ratio, suggesting that LPS significantly impaired immune responsiveness. Taken together, our data provide further support for modulation of immune response during acute LPS administration.

INTRODUCTION

The neuroimmune response is characterized by a bi-directional communication between peripheral immune cells and the central nervous system. Of particular importance during times of infection is the activation of the centrally mediated febrile and corticosterone responses, which aid the innate immune system in the effective clearance of the pathogen while limiting the extent of inflammatory damage (Schobitz et al., 1994; Jiang et al., 1999). Inhibition of these innate immune responses has been shown to significantly affect the morbidity and mortality with infection (Kluger et al., 1998; Jiang et al., 2000; Nadeau and Rivest, 2003).

There is growing evidence that the neonatal immune environment can program the subsequent neuroimmune responsiveness of the adult. A single challenge with the bacterial endotoxin lipopolysaccharide (LPS) during the neonatal period can significantly alter the febrile, neuroendocrine, neurochemical and behavioral responses of the adult (Shanks et al., 2000; Boisse et al., 2004; Ellis et al., 2005; Spencer et al., 2005). A heightened neuroendocrine response, resulting in increased levels of corticosterone, has been shown to underlie the attenuated inflammatory responses to an adult LPS challenge (Ellis et al., 2005), highlighting that programming of these centrally mediated responses by the neonatal immune environment can alter other facets of the innate immune response as well. However LPS, derived from gram-negative bacteria, represents only one type of immune stimulus, which is mediated by the toll-like receptor (TLR)-4 pathways (Tapping et al., 2000).

The aim of the present work was to study the effect of acute activation of the immune system induced by LPS administration.

MATERIALS AND METHODS

Animals

30 male Wistar rats weighing 200-250 g at the start of the experiment were used. The animals were housed in a temperature- and light-controlled room (22°C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC).

Acute LPS administration

The rats were injected, once, with 25µg/kg LPS (lipopolysaccharide from *Escherichia coli*, serotype 0111:B4; Sigma) in 250 µl saline i.p. The saline vehicle received the same doses of physiological saline (0,9% NaCl solution). 7 days postimmunization, whole heparinized blood was collected. To determine the count of leukocyte (µl), blood sample was taken with a leukocytes pipette and diluted (1/20) with the Türk solution. One drop of hemolized blood was transferred onto Neuberg`s haemocytometer, on the counting area of the haemocytometer and than coverslipped. The blood sample was therefore monolayered in a space of 0.1 mm height. The total number of leukocyte was in a $5mm^2$ area was counted and expressed as the number of leukocyte from 1 µl whole blood.

The blood samples were examined in a light microscope KRÜSS model with a magnification of 400 x. The leukocyte formula was determined by means of blood smears stained with May Grünwald-Giemsa. To determine total serum protein, antibody titer and albumin/globulin ratio we used the Weichselbaum method (biuret test for proteins and the technique for albumins).

Corticosterone ELISA

Blood samples (300 µl) were collected in heparinized tubes and immediately centrifuged at 4ºC and plasma was stored frozen at -80ºC until it was assessed. A corticosterone ELISA kit was used to assay the plasma samples for

free corticosterone. The precision and sensitivity of this assay was: interassay variability, 7.8–13.1% CV; intra-assay variability, 6.7–8.0% CV; and sensitivity, 27 pg/ml. Corticosterone concentration is expressed as µg/dl.

Statistical analysis

All data are presented as the mean ± S.E.M. Comparisons between groups were performed using Student's *t*-test. $p<0.05$ was taken as the criterion for significance.

RESULTS AND DISSCUSIONS

1. Effect of acute LPS administration on immune responsiveness of rats

Experimental data were registered 7 days after acute LPS administration. Acute LPS administration, once, significantly impaired the total number of leukocyte ($p<0.05$) (Figure 1.), the total number of lymphocyte ($p<0.05$) (Figure 2.), total serum protein ($p<0.05$) (Figure 3.), antibody titer ($p<0.03$) (Figure 4.) and body weight ($p<0.05$) (Figure 5.) and significantly increased albumin-globulin ratio ($p<0.02$) (Figure 6.) compared to the saline treated-groups. In addition, LPS increased serum corticosterone concentration ($p<0.05$) (Figure 7.), suggesting significant effects on HPAA axis.

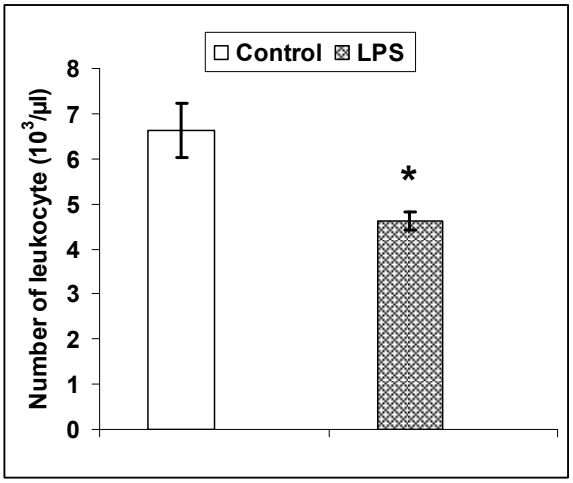

Figure 1. Changes of the total number of leukocyte tested 7 days after acute LPS administration (25µg/kg).
Values are means ± SEM (n=15 per group). *$p<0.05$ vs. Control group.

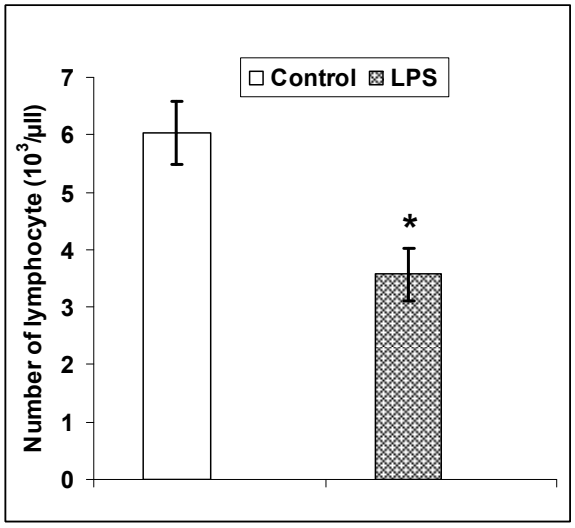

Figure 2. Changes of the total number of lymphocyte tested 7 days after acute LPS administration (25µg/kg).
Values are means ± SEM (n=15 per group). *p<0.05 vs. Control group.

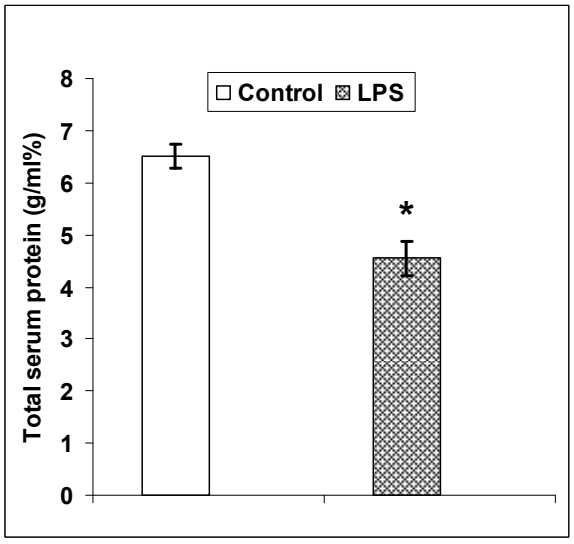

Figure 3. The effect of acute LPS administration (25µg/kg) on the total serum protein level tested 7 days after immunization. Values are means ± SEM (n=15 per group). *p<0.05 vs. Control group.

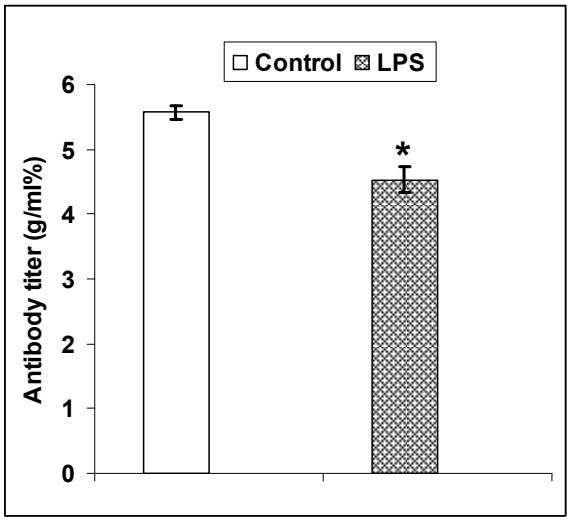

Figure 4. The effect of acute LPS administration (25μg/kg) on the antibody titer tested 7 days after immunization. Values are means ± SEM (n=15 per group). *p<0.03 vs. Control group.

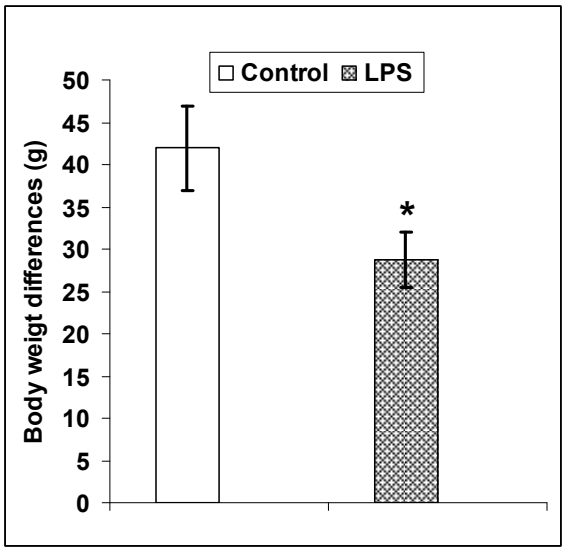

Figure 5. Effect of acute treatment with 25μg/kg LPS on body weight. Values are means ± SEM (n=15 per group). *p<0.05 vs. Control group.

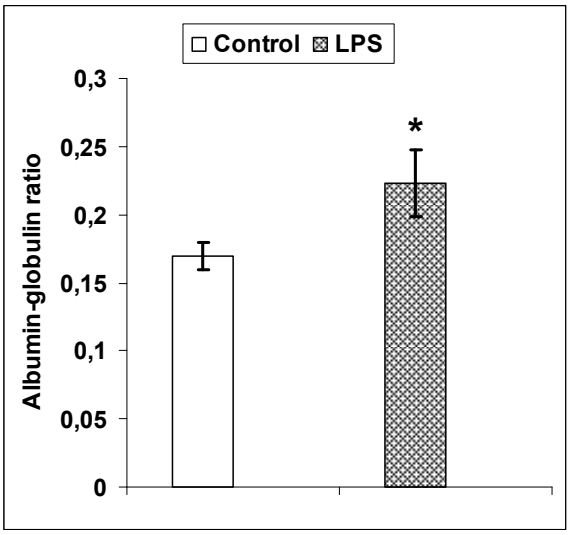

Figure 6. Effect of acute treatment with 25µg/kg LPS on albumin-globulin ratio.
Values are means ± SEM (n=15 per group). *p<0.02 vs. Control group.

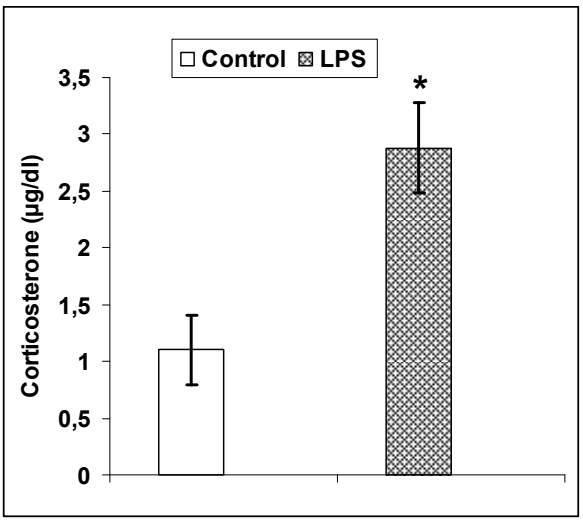

Figure 7. Effect of acute treatment with 25µg/kg LPS on serum concentrations of corticosterone.
Values are means ± SEM (n=15 per group). *p<0.05 vs. Control group.

The present study highlights several important findings regarding the effect of acute LPS administration on immune response modulation of male Wistar rats. First, we have shown that a single dose of LPS, acute administrated could impaired the immune responsiveness of rats, in term of decreased the total number of leukocyte and lymphocyte, total serum protein, antibody

titer and body weight. Second, we have shown that acute LPS administration induced stress, in term of increased serum corticosterone level.

Peripheral administration of low doses of LPS is a very useful model for immune activation. It acts by binding to TLR-4 receptors in various organs, including endothelia. This ultimately results in the synthesis and secretion of IL-1. Not surprisingly, LPS elicits very similar neurochemical, endocrine, and behavioral responses to those of influenza virus and IL-1. It elevates plasma concentrations of ACTH and corticosterone, indicating HPA axis activation. It also increases brain 3-methoxy,4-hydroxyphenylethylene glycol (MHPG, the major brain catabolite of norepinephrine), indicating activation of the brain noradrenergic systems, preferentially in the ventral (diencephalic) system, and increases brain 5-hydroxyindoleacetic acid (5-HIAA, the major catabolite of serotonin), indicating activation of brain serotonergic systems, and increasing brain concentrations of tryptophan (Trp) (Dunn, 1992a,b). Each of these responses resembles those to IL-1. The differences are that the increases in plasma ACTH and corticosterone and the neurochemical responses were significantly slower, perhaps reflecting the delay involved in the induction of IL-1 by LPS. The distinction between the MHPG responses in the dorsal and ventral projection systems is less marked with LPS than with IL-1, and LPS also exhibits a significant activation of dopaminergic systems (i.e., increases in 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) not normally observed with IL-1.

Our results provide further support for involving of LPS on immune response modulation of rats.

CONCLUSIONS

On the basis of our results obtained by LPS administration, we can conclude that peripheral administration of acute LPS impaired immune responsiveness in rats and increased stress response.

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