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Gonadotropin-releasing hormone agonist selectively augments thymopoiesis and prevents cell apoptosis in LPS induced thymic atrophy model independent of gonadal steroids

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ABSTRACT

Lipopolysaccharide (LPS) causes acute thymic atrophy, a phenomenon that has been linked to immune dysfunction and poor survival during sepsis. The systemic response to LPS involves a rise in glucocorticoids and proinflammatory cytokines which contribute greatly to thymic involution and apoptosis. Gonadotropin-releasing hormone (GnRH) analog exerts thymopoietic regulatory effects and possesses immunostimulant properties. We determined whether leuprolide, a GnRH analog can be useful in LPS induced thymic involution and apoptosis. Mice injected with 100 μg of LPS intraperitoneally led to involution of thymus, to decrease of $CD4^+8^+$ thymocyte subset, and to fragmentation of thymic DNA. Leuprolide (100 μg/mouse, s.c.) pretreatment significantly attenuated LPS induced thymic atrophy, and also reduced LPS induced systemic rise in corticosterone levels. The observed effect of leuprolide remained unaffected in castrated and ovariectomized mice. Collectively, leuprolide has protective action independent of gonadal steroids, which was mediated by blunting of the systemic corticosteroid response in LPS induced thymic atrophy model.

1. Introduction

Lipopolysaccharide (LPS) also known as endotoxin, is a normal constituent of the bacterial cell wall of gram negative bacteria [1,2]. It is known that bacterial sepsis is associated with apoptotic cell death in a variety of organs and tissues including the thymus [3,4]. This is accompanied by a classical stress response characterized by activation of the hypothalamic–pituitary–adrenal (HPA) axis [5,6]. During HPA axis activation, there is enhanced synthesis and release of hypothalamic corticotrophin-releasing factor (CRF), resulting in a systemic rise in glucocorticoids and proinflammatory cytokine cascade, and should be contributing to acute thymic involution [7–9]. Acute thymic atrophy in response to endotoxin stress is characterized by specific loss of $CD4^+CD8^+$ double positive (DP) thymocytes and substantially reduced the output of naive T cells, leaving the host potentially vulnerable to new infections [10]. Endotoxin stress induced thymus atrophy is an excellent example of the immune–endocrine relationship, and a useful model to study the mechanisms involved in thymic involution and recovery. Currently, there are no treatments available to protect against acute thymic atrophy or accelerate recovery, thus leaving the immune system compromised during acute stress events. However, it now seems likely that, some CRF receptor antagonists attenuate stress-induced immunosuppression [11].

Gonadotropin releasing hormone (GnRH) is a hypothalamic hormone, which is mainly known for its endocrine effect via the hypothalamic–pituitary–gonadal (HPG) axis [12]. However, endotoxemic stress induced several proinflammatory cytokines, especially IL-1β, IL-6, and TNF-alpha in the brain that disrupts reproductive capability by suppression of GnRH and LH release [13]. It is interesting to note that CRF inhibits GnRH and vice versa [14,15]. Moreover, the behavioral effects of both are seemingly opposite to each other. In addition, GnRH receptor agonist has per se immunostimulant activity, and also ameliorates restrain stress induced immunosuppression [16]. It is known that GnRH analog administration exerts a thymopoietic regulatory effect [17]. Further, the presence of the ligand (GnRH) and receptors (GnRH binding sites) in thymic cells suggests a physiological role of intrathymically produced GnRH in the regulation of T-cell development, in an autocrine or paracrine manner [18,19]. In this regard, literature documents that GnRH analogs influence the absolute and relative thymic weight [20,21] as well as the phenotypic profile of thymocytes in rodents [22,23]. Hence, its effectiveness against LPS induced thymic atrophy needs investigation, particularly because GnRH is reported to completely block hypothalamic release of CRF induced by IL-2 [24].

In the present investigation we addressed this possibility by single or chronic administration of leuprolide, a GnRH agonist, in LPS challenged intact mice. For this purpose a phenotypic analysis and the parameters

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such as relative weight, cellularity of thymus were done. Moreover, we demonstrated the effect of leuprolide on LPS induced alteration in the circulating levels of corticosterone and DNA integrity of thymus. To eliminate the influence of peripheral sex hormones these thymus parameters were also demonstrated in castrated and ovariectomized mice.

2. Materials and methods

2.1. Animals

Inbred Swiss albino mice (6–8 weeks) of either sex weighing about 18–20 g, born and reared in the Animal House of Department of Pharmaceutical Sciences, Nagpur, were used for the present study. The animals were housed in a group of six per cage under a standard light (12:12 h light/dark cycle) and controlled conditions of temperature and humidity (25 ± 2 °C, 55–65%). Animals received standard rodent chow (Poshak Livestock Services, Nagpur, India) and water ad libitum. Studies were approved by the Institutional Animal Ethics Committee, constituted for the purpose of control and supervision of experimental animals by the Ministry of Environment and Forests, Government of India, New Delhi, India. Earlier immunological studies employed Swiss albino mice [25,26], and hence the same has been used in the present study.

2.2. Drugs

Leuprolide acetate and phenol-extracted LPS (L-2880) from *Escherichia coli* (strain O55:B5), were obtained from Sigma-Aldrich (St. Louis, MO, USA). Leuprolide treated mice received 100 μg/mouse by subcutaneous (s.c.) injection, and LPS challenged mice received 100 μg LPS by intraperitoneal (i.p.), which was reconstituted at 1 mg/ml in PBS. The employed dose of leuprolide (100 μg/mouse, s.c.) was based on earlier report and our study, wherein different doses were already tested for influencing the immunological parameters [27]. All other chemicals were of analytical grade.

2.3. Surgical methods

2.3.1. Castration

Mice were castrated as per the procedure described earlier [28]. In brief, after anesthesia with ketamine and xylazine (100 mg/kg and 5 mg/kg, i.m.), animals were placed in supine position, the scrotal skin was disinfected with a 10% povidone–iodine scrub followed by a 70% alcohol wipe. A small scrotal incision was made to expose the testes; all the attachments to testes were ligated and then removed along with surrounding fatty tissue. The incision was sutured with chromic catgut. Sham-operated animals underwent only the scrotal dissection without removal of testes. During the recovery period mice were under antimicrobial cover of cefotaxime (50 mg/kg/day, s.c.).

2.3.2. Ovariectomy

Ovariectomy was performed as per the procedure described earlier [29]. In brief, the surgery was performed under anesthesia with ketamine and xylazine (100 mg/kg and 5 mg/kg, i.m.); the lumbar dorsum of mouse was shaved bilaterally and exposed skin was prepared for surgery by a 10% povidone–iodine scrub followed by a 70% alcohol wipe. Each ovary was accessed via a small paravertebral incision between the lower rim of the rib cage and the upper pelvic ridge. The oviduct and adjacent blood vessels were ligated using a single suture. The ovaries were then removed; the incisions were closed in two layers by chromic catgut. Sham-operated animals underwent only the dissection without removal of ovaries. During the recovery period, mice were under antimicrobial cover of cefotaxime (50 mg/kg/day, s.c.).

2.4. Experimental design

2.4.1. Effect of LPS on thymic involution and cell apoptosis

A group of mice were given vehicle saline (10 mg/kg, i.p.) or LPS (100 μg/mouse, i.p.), and three mice per group were euthanized on days 1, 3, 7, 10, 14, 20, and 30 to monitor thymopoiesis. Thymus glands were removed for phenotypic analysis, and determination of the parameters such as relative weight, cellularity and DNA integrity.

2.4.2. Influence of leuprolide pretreatment on thymus parameters and corticosterone levels in LPS challenged mice

A group of mice received saline (10 ml/kg, s.c.) or leuprolide (100 μg/mouse, s.c.) 5 min prior to the administration of single dose of saline (10 ml/kg, i.p.) or LPS (100 μg/mouse, i.p.). Blood was obtained from the retro-orbital venous plexus at sequential time points (1, 2, 4, 6, 12 h) during the first 12 h after LPS challenged with or without leuprolide. Animals showing anemia were discarded, and the remaining were used for statistical analysis (n = 3–4 for each time point). Serum was isolated by centrifugation for 20 min at 2000 ×*g* and stored at −20 °C until thawed for analysis of corticosterone. Administration of

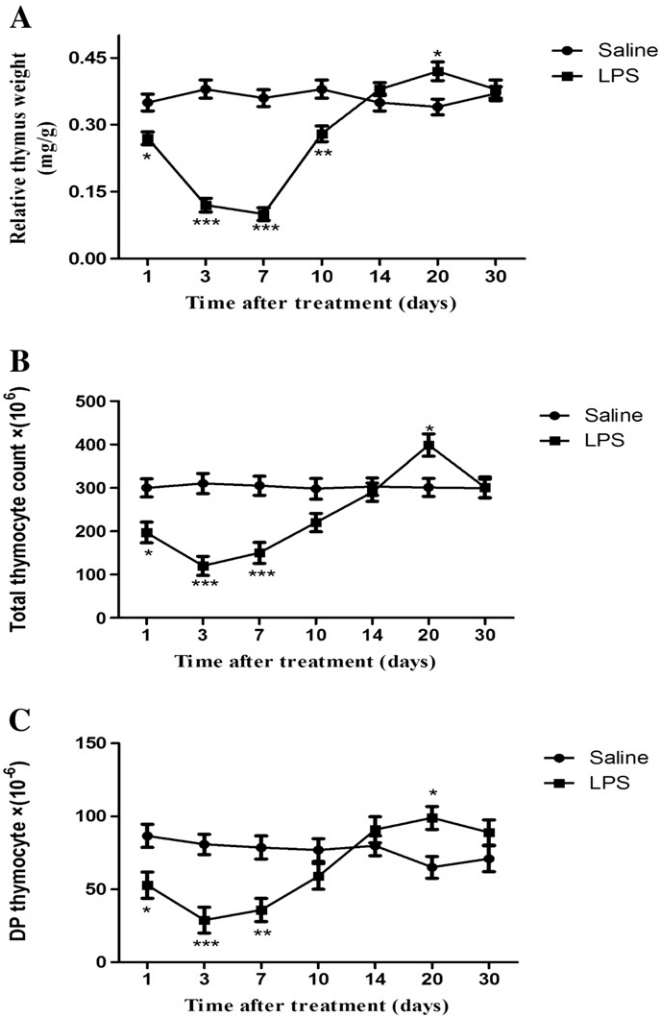

Fig. 1. Influence of a single dose administration of *E. coli* LPS on thymic homeostasis. Groups of mice were treated with saline (10 mg/kg, i.p.) or LPS (100 μg i.p.) on day 0, and three mice per group were euthanized on days 1, 3, 7, 10, 14, 20, and 30 to monitor thymopoiesis. Relative thymus weights (A), thymic cellularity (B) and CD4/CD8 DP thymocytes number of thymus tissue (C) were determined. Values are expressed as the mean ± SEM at each harvest time. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. respective control saline treated group (n = 3) (two-way ANOVA followed by Bonferroni test for multiple comparisons).