Journal of Infection (2014) 69, 113–122

BIA
British Infection Association

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Glucocorticoid receptor function is decreased in neutrophils during endotoxic shock

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Accepted 11 March 2014
Available online 19 March 2014

KEYWORDS
Lipopolysaccharide;
Endotoxin;
Glucocorticoid receptor;
Sepsis;
Dexamethasone

Summary *Objectives:* It remains unclear whether glucocorticoid treatment can improve the outcome of sepsis. The aim of the present study was to investigate if glucocorticoid receptor (GR) expression and function is impaired in lipopolysaccharide (LPS) induced shock, and whether the time point for start of glucocorticoid treatment affects the outcome.
Methods: Male C57BL/6J mice were administered LPS i.p. and GR expression and binding ability in blood and spleen leukocytes were analysed by flow cytometry. GR translocation was analysed using Image Stream technique. The effect of dexamethasone treatment started 2 h before or 2, 12 or 36 h after LPS administration on survival was studied.
Results: Despite increased GR expression in neutrophils after LPS administration, the GR binding capacity was reduced. In addition, GR translocation was decreased in neutrophils and T lymphocytes from endotoxic mice at 12 h compared to control animals. Dexamethasone treatment improved survival only when started early (2 h) after LPS administration.
Conclusion: The decreased glucocorticoid responsiveness displayed by neutrophils, in combination with their increased numbers, may explain why survival is increased only when dexamethasone treatment is given early during LPS induced shock.

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0163-4453/$36
http://dx.doi.org/10.1016/j.jinf.2014.03.011

Introduction

Despite recent intense research and advances in therapy the mortality in severe sepsis and septic shock remains high.[1] An overproduction of pro-inflammatory mediators and/or an inadequate anti-inflammatory response is likely to play a critical role in the development of multiple-organ dysfunction syndrome (MODS), and correlated mortality.[2,3] During the last decades, treatment with general anti-inflammatory agents as an attempt to compensate for a putative inadequate cortisol response has been pursued with varying success.[4] Thus, much controversy still surrounds the topic of glucocorticoid treatment in sepsis and large multicentre trials have shown contradictive results regarding the effect of glucocorticoid treatment of sepsis.[5,6]

Adrenal glucocorticoid production in sepsis is the result of the pathogen-induced cytokine response activating the hypothalamic–pituitary–adrenal (HPA) axis.[7] Glucocorticoids exert immunomodulatory effects through intracellular glucocorticoid receptors (GR). The two main human isoforms of GR, GRα and GRβ, originate from alternative splicing of the same exon.[8,9] The GRβ isoform is considered to be pro-inflammatory in the sense that it inhibits the anti-inflammatory effects of GRα, by competing for the glucocorticoid response element (GRE) on DNA.[10] It has previously been suggested that the balance of GRα and GRβ play an important role in glucocorticoid sensitivity in sepsis, either via a downregulation of GRα or relative upregulation of GRβ.[11,12] Recently, GRβ was also found to be present in mice,[13] making this species attractive for studies on the immunomodulatory effects of glucocorticoids. To model the systemic immune response seen in systemic inflammatory response syndrome (SIRS) and septic shock and activate the HPA axis, endotoxin from gram-negative bacteria (lipopolysaccharide, LPS) can be used.[14]

In earlier experiments, using our gram-positive sepsis model in mice, we have shown that the expression and function of GR is progressively decreased during *Staphylococcus aureus* infection and that dexamethasone treatment is only beneficial when administered early in the disease course.[15] Our findings prompted us to investigate the effect of LPS induced shock on the GR expression and its glucocorticoid binding ability, and whether time is an important factor also for glucocorticoid treatment in this model of LPS induced shock. We hypothesized that failure to respond to glucocorticoid therapy may be caused by decreased expression and/or function of GR during endotoxic shock.

Materials and methods

Mice

Male C57BL/6J mice, 6–8 weeks old, were obtained from Charles River Laboratories (Wilmington, MA) and maintained in the animal facility at the Department of Rheumatology and Inflammation Research at Gothenburg University under standard light and temperature conditions. Mice were fed soy-free laboratory chow and tap water *ad libitum*. Permission from the local animal research ethics committee, in accordance with national animal welfare legislation, was obtained for all experiments.

Endotoxic shock model

LPS from *Escherichia coli* (O111:B4) in PBS was administrated by intraperitoneal (i.p.) injection at a dosage of 10 mg/kg. The LPS dose was chosen from an initial dose finding experiment with three doses of 5, 10 and 25 mg/kg ($n = 18$, data not shown). All animals were weighed daily and systemic inflammation was visually graded by inspection of activity, fur condition and breathing frequency. Weight development was used to monitor general health and dehydration, with weight loss as a strong indicator of the animals failing to maintain a fluid balance by ceasing to eat and drink. If a mouse was judged too ill to survive for another 12 h or if it was neurologically affected (mono- or hemi-plegia), it was euthanized and defined as dead due to septic shock. The animals were anesthetised with a mixture of Ketalar (Pfizer AB, Sollentuna, Sweden) and Dormitor Vet (Orion Pharma, Espoo, Finland) before they were culled. Cells from blood and spleens were isolated and analysed immediately by means of flow cytometry. Kidneys were snap frozen in liquid nitrogen and stored at −70 °C until RNA preparation.

Dexamethasone treatment

For analysis of GR expression and function during endotoxic shock, mice were injected with LPS and compared with healthy mice. For the dexamethasone treatment experiment, mice were administered LPS and treated with dexamethasone at different starting time points in comparison to the control group, only given LPS. Dexadreson (Intervet AB, Sollentuna, Sweden) 0.05 mg/kg was administered i.p. once daily for 4 consecutive days starting at 2 h before or 2, 12 or 24 h after LPS administration. The protocol is displayed in SI Fig. 1. The dose was chosen from literature data.[16]

Collection of tissue samples and cell preparation

Single cell suspensions were prepared from spleens by homogenisation in PBS using a 70 μm nylon wool sieve. Blood and spleen cells were subjected to erythrocyte lysis using Tris-buffered 0.83% NH_4Cl, pH 7.3, for 8 min at room temperature, washed with PBS, filtered through a 35 μm cell strainer (BD Falcon, BD, Franklin Lakes, NJ, USA) before the total number of leukocytes was determined using an automated cell counter (Sysmex KX-21N, Kobe, Japan).

Surface staining for flow cytometry

A total of 5×10^5 cells were added to each well of a polypropylene conical 96-well plate (Thermo Fisher Sci, Rochester, NY, USA) and stained with fluorochrome conjugated antibodies binding to cell surface markers after Fc-blockage with anti-CD16/CD32. The antibodies used were; anti-CD4 v500 (BD Horizon, BD Biosciences), anti-CD3 PE and APC (145-2C11), anti-CD19 PerCP (6D5), anti-F480