Brain, Behavior, and Immunity xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Cortisol-dependent stress effects on cell distribution in healthy individuals and individuals suffering from chronic adrenal insufficiency

Ashley M. Geiger [a], Kenneth P. Pitts [a], Joachim Feldkamp [b], Clemens Kirschbaum [c], Jutta M. Wolf [a,*]

[a] Brandeis University, Waltham, MA, United States
[b] Klinikum Bielefeld, Bielefeld, Germany
[c] Technische Universität Dresden, Dresden, Germany

ARTICLE INFO

Article history:
Received 16 March 2015
Received in revised form 10 July 2015
Accepted 11 July 2015
Available online xxxx

Keywords:
Cell trafficking
Chronic adrenal insufficiency
Psychosocial stress
Cortisol
Catecholamines

ABSTRACT

Chronic adrenal insufficiency (CAI) is characterized by a lack of glucocorticoid and mineralocorticoid production due to destroyed adrenal cortex cells. However, elevated cortisol secretion is thought to be a central part in a well-orchestrated immune response to stress. This raises the question to what extent lack of cortisol in CAI affects stress-related changes in immune processes.

To address this question, 28 CAI patients (20 females) and 18 healthy individuals (11 females) (age: 44.3 ± 8.4 years) were exposed to a psychosocial stress test (Trier Social Stress Test: TSST). Half the patients received a 0.03 mg/kg body weight injection of hydrocortisone (HC) post-TSST to mimic a healthy cortisol stress response. Catecholamines and immune cell composition were assessed in peripheral blood and free cortisol measured in saliva collected before and repeatedly after TSST.

CAI patients showed norepinephrine (NE) stress responses similar to healthy participants, however, epinephrine (E) as well as cortisol levels were significantly lower. HC treatment post-TSST resulted in cortisol increases comparable to those observed in healthy participants (interaction effects – NE: $F = 1.05$, $p = .41$; E: $F = 2.56$, $p = .045$; cortisol: $F = 13.28$, $p < .001$). Healthy individuals showed the expected pattern of stress-related early lymphocyte increase with subsequent decrease below baseline. The opposite pattern was observed in granulocytes. While exhibiting a similar initial increase, lymphocytes kept increasing over the following 2 h in untreated patients. HC treatment buffered this effect (interaction effects – lymphocyte%: $F = 7.31$, $p < .001$; granulocyte%: $F = 7.71$, $p < .001$).

Using CAI in humans as a model confirms cortisol's central involvement in post-stress lymphocyte migration from blood into immune-relevant body compartments. As such, future studies should investigate whether psychosocial stress exposure may put CAI patients at an increased health risk due to attenuated immune responses to pathogens.

1. Introduction

Elevated cortisol and catecholamine secretion are thought to be a central part in a well-orchestrated immune response to stress (Dhabhar et al., 2012). While studied extensively in animals, it is less clear to what extent the inability to mount a cortisol response affects stress-related changes in immune processes in humans. The current study proposes chronic adrenal insufficiency (CAI) as a model for assessing the role of cortisol in stress-related immune cell trafficking.

1.1. Stress affects immune cell distribution

Psychosocial stress is associated with increased activity of the hypothalamus–pituitary–adrenal (HPA) axis as well as the sympathetic nervous system (SNS) (Dickerson and Kemeny, 2004). The major end products of these systems are the hormones cortisol (HPA axis) and epinephrine and norepinephrine (SNS). Stress-induced release of these mediators has a wide range of effects on somatic systems, which generally are thought of as well-orchestrated and thus primarily protective (Sapolsky et al., 2000). One immune process of particular interest is the migration of immune cells across vascular endothelium (i.e., cell trafficking), for example, from blood or lymph fluid into tissue or a site of inflammation.

* Corresponding author at: Brandeis University, 415 South St., MS 062, Waltham, MA 02453, United States.
E-mail address: jmw@brandeis.edu (J.M. Wolf).

http://dx.doi.org/10.1016/j.bbi.2015.07.010
0889-1591/

Stress interacts with this process on multiple levels. The fast release of catecholamines occurring within seconds after onset of a stressor increases the number of circulating natural killer (NK)-cells and granulocytes. This process is thought to ensure fast transportation of cells central for innate immunity to sites of tissue damage, thereby reducing the risk for infections (Dhabhar et al., 2012; Benschop et al., 1996; Sanders, 2006). Stress-induced changes in glucocorticoids induce a pronounced decrease in lymphocytes, indicating migration of lymphocytes out of blood and into immune compartments or sites of inflammation (Dhabhar et al., 2012). Importantly, cell trafficking is a clinically relevant immune function. For example, changes in immune cell composition patterns observed in response to the stress of undergoing surgery have been shown to predict speed of recovery post-surgery (Rosenberger et al., 2009).

1.2. Methodological considerations

To determine whether cortisol is sufficient or necessary for stress-related cell trafficking patterns, lack of hormone and subsequent hormone replacement is the approach of choice (Sapolsky et al., 2000). Adrenalectomy studies have been helpful in determining the extent to which a lack of cortisol response affects the stress–immune relationship. For example, adrenalectomized animals do not show a stress-induced decline in leukocytes compared to intact animals (Dhabhar and McEwen, 1999), while administering corticosterone to these animals restores the response (Dhabhar et al., 1995). However, adrenalectomy removes the entire adrenal gland, including the medulla. This not only prevents the production of glucocorticoids, but also the local production of catecholamines (Dhabhar and McEwen, 1999), making it difficult to tease apart the effects of a missing cortisol response from altered catecholamine responses. In addition to adrenalectomy studies, glucocorticoid synthesis inhibitors and receptor antagonists have been used to isolate the role of glucocorticoids in stress induced cell trafficking (Glover et al., 2011; Dhabhar et al., 1996). However, these approaches acutely remove glucocorticoids from the system and thus do not separate the impact of missing cortisol responsivity from effects of missing basal glucocorticoid levels. Furthermore, they do not allow for investigation of more health-relevant long-term adaptations to a chronic lack of glucocorticoid increases in response to physical and psychosocial stress.

1.3. The current study: Chronic adrenal insufficiency

The current study aimed at finding a model for studying the role of cortisol in stress-related immune cell trafficking that addresses some of the methodological gaps from alternative approaches outlined above. Investigating patients suffering from chronic adrenal insufficiency (CAI) is proposed as a promising approach.

Patients with CAI do not produce any cortisol due to destroyed adrenal cortex cells and thus receive glucocorticoid (and mineralocorticoid) replacement (Ten et al., 2001; Betterle et al., 2002; Oelkers, 1996; Arlt and Allolio, 2003). This therapy only substitutes basal cortisol levels, while no provisions are made for additional doses during stress. As a result, CAI patients are specifically lacking the ability to mount a cortisol stress response. The first aim of the current study was therefore to confirm the supposed lack of endocrine stress responses in patients with CAI. The second aim was to investigate the effects of missing cortisol stress responses on immune cell trafficking. To distinguish between sufficient and necessary effects of cortisol, half of the CAI patients were treated with 0.03 mg/kg hydrocortisone *i.v.* to pharmacologically mimic cortisol stress responses.

2. Methods

2.1. Participants

A total of 36 patients with CAI and 21 age- and gender-matched healthy participants (HP) were investigated. We excluded five participants for missing one or more blood samples (4 CAI patients), two for missing saliva samples (one CAI patient), three for being under-age (two CAI patients), and one patient for abnormally high norepinephrine levels at baseline (>4 SD) and throughout the study protocol (2–3 SD above the mean). Hence, the final sample consisted of 28 individuals with CAI (20 females) and 18 controls (11 females) with a mean age of 44.33 years (SD = 8.36 years). Based on presence of autoantibodies (adrenocortical autoantibodies, steroid cell antibodies) (Betterle et al., 2002; Peterson et al., 2000) or co-morbidities fulfilling criteria for classification to Autoimmune Polyglandular Syndrome (APS) type 1 or type 2 (Neufeld et al., 1981), 19 patients were diagnosed with autoimmune CAI (67.9%). In four patients, the cause for CAI was former Cushing's disease and five patients did not provide sufficiently detailed information for differential diagnosis.

Half of the CAI patients were randomly assigned to receive 0.03 mg/kg hydrocortisone (HC) *i.v.* (Sigma, Berlin) after a psychosocial stress test (CAI-HC: $n = 14$), while the remaining $n = 14$ patients as well as healthy participants (HP) received an injection of 4 mL saline (NaCl). The CAI-HC and CAI-NaCl groups each consisted of 10 females and 4 males and the gender make-up of the three groups did not significantly differ ($\chi^2 = .53$, $p = .77$). The three groups also did not differ in age ($F_{2,43} = .36$, $p = .70$; CAI-HC: mean = 43.81, SD = 8.5; CAI-NaCl: mean = 44.87, SD = 7.9; HP-NaCl: mean = 45.06, SD = 9.8) or body mass index (BMI: $F_{2,43} = 1.31$, $p = .28$; CAI-HC: mean = 23.89, SD = 4.3; CAI-NaCl: mean = 23.98, SD = 2.6; HP-NaCl: mean = 25.40, SD = 4.6). Lastly, the two patient groups did not differ in etiology of CAI ($\chi^2 = 4.16$, $p = .25$) nor the number of years with the disease (CAI-HC: mean = 7.42 years, SD = 7.22; CAI-NaCl: mean = 10.14 - years, SD = 9.90; $\chi^2 = 19.29$, $p = .31$). The ethics committee of the University of Düsseldorf approved the study protocol.

2.2. Procedures

Patients and healthy participants were recruited from across Germany (travel distance: 287 ± 157 km; $t = 1.69$, $p = .20$), with patients being referred by the study's endocrinologist (J.F.). Participants arrived in the laboratory at 1 PM and were examined for past or current health problems by the study's physician. CAI participants were asked to postpone their second glucocorticoid replacement dose usually taken around 2 PM (mean = 13:49 h, SD = 1h 57 min) to avoid cortisol levels pre-stressor being significantly higher than in healthy participants. After obtaining written consent, a catheter was inserted into participants' preferred arm. After a 45-min adjustment period allowing stress from catheter insertion to subside, a first blood (2.7 mL and 9 mL EDTA Monovettes, Sarstedt, Nümbrecht, Germany) and saliva (Salivette, Sarstedt, Nümbrecht, Germany) sample was collected. Subsequently, participants were exposed to the Trier Social Stress Test (TSST). Shortly after TSST exposure, participants received either a hydrocortisone or placebo injection in a double-blind design. Additional blood and saliva samples were collected 1, 10, 20, 30, 45, 60, 90, and 120 min after stress exposure (see Fig. 1).

Please cite this article in press as: Geiger, A.M., et al. Cortisol-dependent stress effects on cell distribution in healthy individuals and individuals suffering from chronic adrenal insufficiency. Brain Behav. Immun. (2015), http://dx.doi.org/10.1016/j.bbi.2015.07.010