Brain, Behavior, and Immunity xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Full-length Article

# Glucocorticoids enhance the *in vivo* migratory response of human monocytes ☆

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ARTICLE INFO

*Article history:*
Received 9 September 2015
Received in revised form 6 January 2016
Accepted 10 January 2016
Available online xxxx

*Keywords:*
Cortisol
Stress
Monocyte
Macrophage
CCR2
CX3CR1
IκBα

ABSTRACT

Glucocorticoids (GCs) are best known for their potent anti-inflammatory effects. However, an emerging model for glucocorticoid (GC) regulation of *in vivo* inflammation also includes a delayed, preparatory effect that manifests as enhanced inflammation following exposure to an inflammatory stimulus. When GCs are transiently elevated *in vivo* following exposure to a stressful event, this model proposes that a subsequent period of increased inflammatory responsiveness is adaptive because it enhances resistance to a subsequent stressor. In the present study, we examined the migratory response of human monocytes/macrophages following transient *in vivo* exposure to stress-associated concentrations of cortisol. Participants were administered cortisol for 6 h to elevate *in vivo* cortisol levels to approximate those observed during major systemic stress. Monocytes in peripheral blood and macrophages in sterile inflammatory tissue (skin blisters) were studied before and after exposure to cortisol or placebo. We found that exposure to cortisol induced transient upregulation of monocyte mRNA for CCR2, the receptor for monocyte chemotactic protein-1 (MCP-1/CCL2) as well as for the chemokine receptor CX3CR1. At the same time, mRNA for the transcription factor IκBα was decreased. Monocyte surface expression of CCR2 but not CX3CR1 increased in the first 24 h after cortisol exposure. Transient exposure to cortisol also led to an increased number of macrophages and neutrophils in fluid derived from a sterile inflammatory site *in vivo*. These findings suggest that the delayed, pro-inflammatory effects of cortisol on the human inflammatory responses may include enhanced localization of effector cells at sites of *in vivo* inflammation.



## 1. Introduction

Glucocorticoids (GCs) are widely understood to suppress inflammation through regulation of signaling pathways in leukocytes and other effector cells. Until recently, these potent and clinically important effects of GCs have obscured results from earlier research that demonstrated GC support and even stimulation of *in vivo* defense mechanisms (Ingle, 1952). An emerging model for GC regulation of defense mechanisms includes not only their well-known anti-inflammatory properties but also a teleological conception in which GCs can enhance the *in vivo* response to external stressors. Evidence for GC enhancement of inflammation was described as early as 1954 by Selye (Selye, 1954), was re-introduced into the clinical literature in 1984 (Munck et al., 1984), and has since been expanded by subsequent research (Barber et al., 1993; Yeager et al., 2004; Frank et al., 2009; Sorrells and Sapolsky, 2007; Busillo and Cidlowski, 2013; Kamisoglu et al., 2014; Cruz-Topete and Cidlowski, 2015). Time is a critical variable. Concurrent exposure of immune effector cells to GCs engenders the two best-known properties of GCs: (1) At low 'basal' concentrations, a 'permissive' GC effect supports other metabolic and inflammatory processes so that they proceed normally (Ingle, 1950; Udelsman et al., 1986). The absence of this GC activity leads to the Addisonian crisis first described in the 19th century (Addison, 1849). (2) At higher concentrations of GCs, when concurrent with systemic stress, the well-known anti-inflammatory effects of GCs are observed and act to prevent damage from an excessive inflammatory response to an imposed stimulus (Munck and Náray-Fejes-Tóth, 1992). However, markedly different effects can be observed when *transient* (hours) *in vivo* exposure to elevated GC concentrations is followed by a return

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http://dx.doi.org/10.1016/j.bbi.2016.01.004
0889-1591/

to normal GC concentrations: (1) 'Preparatory' effects are observed in which cells, especially immune effector cells, undergo transcriptional and phenotypic changes that prime them for an enhanced response to a subsequent immune stimulus (Barber et al., 1993; Frank et al., 2009; Dhabhar, 2002; Dhabhar et al., 1996; Dhabhar, 2014; Zhang and Daynes, 2007). These preparatory effects have been reported for both innate (especially following TLR4 ligation (Frank et al., 2009; Sorrells and Sapolsky, 2007; Busillo and Cidlowski, 2013; Zhang and Daynes, 2007) and adaptive immune cells (Busillo and Cidlowski, 2013; Dhabhar, 2002; Hermoso et al., 2004; Sorrells and Sapolsky, 2010; Busillo et al., 2011; Frank et al., 2013). (2) Stimulation of effector cell responses is observed if an external inflammatory stimulus is applied during the preparatory period (Barber et al., 1993; Kamisoglu et al., 2014; Dhabhar, 2014; Sorrells et al., 2013; Yeager et al., 2009). Preparatory and stimulatory effects typically manifest beginning several hours after a transient exposure to elevated GCs (and a subsequent return to normal GC concentrations) and may last for up to a week (Barber et al., 1993; Zhang and Daynes, 2007). These delayed preparatory and stimulatory effects appear to enhance an organism's resistance to a subsequent stressful event (infection after injury, for example). The teleological components of this model therefore include the initial response of an organism to an external stimulus resulting in elevated GCs that enable defense mechanisms to proceed optimally by a permissive effect and then act to prevent an exaggerated inflammatory response from damaging normal tissue by way of GC anti-inflammatory effects. The following period of enhanced inflammatory responsiveness appears to be an adaptive effect that promotes resistance to subsequent stressors.

*In vivo* experiments in humans and animals support this concept. Barber et al. showed that a 6-h *in vivo* exposure of healthy humans to pharmacological doses of cortisol induces a substantial increase in their pro-inflammatory response to a subsequent challenge with bacterial endotoxin (Barber et al., 1993). This same effect can be reproduced *in vivo* by "stress cortisol" concentrations that are similar to those observed during a systemic stress such as surgery (Yeager et al., 2009). Animal experiments yield similar results. Exposure of rats to the sterile stress of a skin burn induces a subsequent state of resistance to an otherwise lethal inoculation with gram-negative bacteria and enhances the pro-inflammatory response to a sublethal injection of bacterial lipopolysaccharide (Maung et al., 2008; Paterson et al., 2003). Systemic stress can also induce expression of Toll-like receptors 4 (TLR4) and 2 (TLR2) on macrophages (Hermoso et al., 2004; Paterson et al., 2003) suggesting that stress may increase resistance to bacterial infection through up-regulation of the pro-inflammatory response to bacterial pathogen-associated molecular patterns (PAMPs). Enhanced activation of anti-viral responses has also been reported in mice subjected to repeated social stress (Powell et al., 2011). In these studies, the relative contribution of GCs and other stress responding hormones, specifically catecholamines, has still to be clarified (Tarr et al., 2012). GCs have also been shown to enhance neuroinflammatory responses in animals in association with an upregulation of high-mobility group box-1 (HMGB1), which in turn primes the inflammasome response through TLR2/TLR4 signaling (Frank et al., 2015). *In vitro*, murine bone marrow-derived macrophages (Zhang and Daynes, 2007; Lloberas et al., 1998) and peritoneal macrophages (Schmelzer et al., 2008) show a similar response pattern of a GC-induced increase in the pro-inflammatory response to PAMPs.

Until recently, there have been few reports on the cellular and molecular events that are associated with GC stimulation of human immune responses. In the experiments described here we demonstrate that GCs can enhance key components of the human monocyte/macrophage migratory response.

## 2. Materials and methods

### 2.1. Clinical protocols

Clinical protocols were approved by the Dartmouth College Committee for the Protection of Human Subjects (Institutional Review Board) and written informed consent was obtained from all participants. Subjects were healthy non-obese males or females, non-smokers, taking no chronic medication and with no history of illness or injury within the 3 months prior to study.

#### 2.1.1. In vivo stress cortisol exposure

Participants were exposed to stress-associated concentrations of cortisol for 6 h initially by the intravenous and subsequently the oral route. Intravenous cortisol (hydrocortisone; SoluCortef, Upjohn) was administered at a rate of 1.5 ug/kg/min, a dose that we have previously shown increases plasma cortisol to concentrations that are observed when humans are exposed to a systemic stress such as major surgery (Yeager et al., 2005) or endotoxemia (Yeager et al., 2009). Oral cortisol (Cortef®, Pfizer) was administered as a 30 mg oral loading dose followed by 10 mg orally every hour for 5 subsequent doses leading to similar increases of *in vivo* cortisol concentrations as described below.

#### 2.1.2. Sterile inflammation

Healthy volunteers ($n = 7$) participated in the following protocol: From 0800 to 0930, eight 8 mm diameter blisters were raised on the volar aspect of a participant's forearm using a commercially available device (NP-4 Negative Pressure Instrument, Electronic Diversities, Finksburg, MD). The device has a metal plate with 8 mm diameter holes that is placed against the skin and attached to a negative pressure chamber with programmable suction. A negative pressure of −250 mmHg is applied to the chamber over the skin. After 90 min, blisters are raised on the skin via dermo-epidermal separation through the lamina lucida (Willsteed et al., 1991). The procedure is painless. Blister roofs were then removed in a sterile manner and a sterile plastic template placed over the blisters. The template contains 1.0 cm diameter cones that are designed so that one cone fits over each blister base. The cones (~0.8 ml volume) were filled with sterile saline alone or with sterile saline containing 10 ng/ml human CCL2 (PeproTech, #300-04) and taped to the arm with a pressure bandage to maintain a seal with the skin. One subject did not receive CCL2 in the chamber fluid. The chamber template was removed on the following day at 10:00 AM and transmigrated leukocytes in chamber fluid were collected and the blister base covered with a sterile dressing. Healing takes place in 5–7 days. Participants served as their own control and received 2 separate treatments immediately after the blisters were created. They first received a control placebo treatment from 1000 to 1600 h followed by collection of transmigrated leukocytes in chamber fluid 18 h later on the following day. After a rest period of 3–14 days, participants then received a 6-h cortisol exposure. On these treatment days, blisters were raised on the forearm opposite the one used on the control day. The 6-h control (placebo) and experimental (cortisol) exposures were deliberately timed so that blisters would be present during the period of GC induced monocytopenia (Fauci and Dale, 1974; Calvano et al., 1987) and to coincide with the expected period of peak GC induced inflammatory responses 12 to 24 h after GC exposure (Barber et al., 1993; Yeager et al., 2009).

### 2.2. Laboratory protocols

#### 2.2.1. Salivary cortisol measurements

Saliva samples were collected (Salivette, Sarstedt #51.1534) at the time points indicated. Samples were stored at −80 °C until

Please cite this article in press as: Yeager, M.P., et al. Glucocorticoids enhance the *in vivo* migratory response of human monocytes. Brain Behav. Immun. (2016), http://dx.doi.org/10.1016/j.bbi.2016.01.004