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Greater inflammatory activity and blunted glucocorticoid signaling in monocytes of chronically stressed caregivers

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ABSTRACT

Chronic stress is associated with morbidity and mortality from numerous conditions, many of whose pathogenesis involves persistent inflammation. Here, we examine how chronic stress influences signaling pathways that regulate inflammation in monocytes. The sample consisted of 33 adults caring for a family member with glioblastoma and 47 controls whose lives were free of major stressors. The subjects were assessed four times over eight months. Relative to controls, caregivers' monocytes showed increased expression of genes bearing response elements for nuclear-factor kappa B, a key pro-inflammatory transcription factor. Simultaneously, caregivers showed reduced expression of genes with response elements for the glucocorticoid receptor, a transcription factor that conveys cortisol's anti-inflammatory signals to monocytes. Transcript origin analyses revealed that CD14+/CD16− cells, a population of immature monocytes, were the predominate source of inflammatory gene expression among caregivers. We considered hormonal, molecular, and functional explanations for caregivers' decreased glucocorticoid-mediated transcription. Across twelve days, the groups displayed similar diurnal cortisol profiles, suggesting that differential adrenocortical activity was not involved. Moreover, the groups' monocytes expressed similar amounts of glucocorticoid receptor protein, suggesting that differential receptor availability was not involved. In *ex vivo* studies, subjects' monocytes were stimulated with lipopolysaccharide, and caregivers showed greater production of the inflammatory cytokine interleukin-6 relative to controls. However, no group differences in functional glucocorticoid sensitivity were apparent; hydrocortisone was equally effective at inhibiting cytokine production in caregivers and controls. These findings may help shed light on the mechanisms through which caregiving increases vulnerability to inflammation-related diseases.

1. Introduction

Prospective studies show that chronic psychological stress undermines health. People who have persistent marital difficulties, lose their jobs and struggle to find work, or assume care for a terminally ill relative are prone to developing new health problems and worsening of existing ones (Christakis and Allison, 2006; Dupre et al., 2012; Ji et al., 2012; Matthews and Gump, 2002; Schulz and Beach, 1999; Schulz et al., 2003). The health consequences of chronic stress emerge in both mental and physical illnesses, with the most pronounced effects in depression, respiratory infections, HIV/AIDS, and cardiovascular disease (Cohen et al., 2007). Research shows that "nonresolving" inflammation plays a role in the pathogenesis and expression of all these conditions (Libby et al., 2011; Miller et al., 2009a; Nathan and Ding, 2010; Pace and Heim, 2011; Scrivo et al., 2011). Drawing on these insights, researchers have begun elucidating how chronic stress affects inflammation and its regulation by the immune, nervous, and endocrine systems (Irwin and Cole, 2011; Raison et al., 2006; Sternberg, 2006).

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Recent gene expression profiling studies have revealed a "transcriptional fingerprint" of chronic stress in the monocytes of humans (Cole et al., 2011; Miller et al., 2008; O'Donovan et al., 2011), the immune cells that initiate and sustain many inflammatory responses. Bioinformatic analyses indicate this "conserved transcriptional response to adversity" is characterized by three predominate themes. First, chronic stress downregulates transcriptional activity mediated by interferon response factors. Second, chronic stress upregulates activity of pro-inflammatory transcription control pathways, especially those mediated by members of the nuclear factor-kappa B (NF-κB) family. Finally, chronic stress downregulates transcriptional activity mediated by the glucocorticoid receptor (GR), the apparatus that propagates cortisol signals to the genome of target cells. Cortisol has well-known anti-inflammatory properties, partly mediated through GR inhibition of NF-κB signaling (Beck et al., 2009; Busillo and Cidlowski, 2013). For this reason, researchers believe the transcriptional fingerprint reflects acquired glucocorticoid insensitivity. According to this hypothesis, chronic stress weakens the usual regulatory constraints on monocyte pro-inflammatory activity via diminution of cortisol-mediated signaling through GR (Cole et al., 2007; Miller et al., 2008, 2009b; Miller, 2008).

These findings have been substantiated in *ex vivo* functional studies, where monocytes are stimulated with bacterial products in the presence of cortisol, and production of pro-inflammatory cytokines is monitored. Under these conditions, chronically stressed individuals produce more inflammatory cytokines than controls, and their cells are less sensitive to inhibition by cortisol (Cohen et al., 2012; Miller et al., 2002; Rohleder et al., 2009; Rohleder, 2012). This stress-related diminution of cortisol sensitivity has implications for the pathophysiology of neuropsychiatric conditions like depression, chronic fatigue, and PTSD (Raison and Miller, 2003), as well as common diseases like upper respiratory infection (Cohen et al., 2012).

Despite this progress, little is known about the mechanism(s) through which chronic stress reduces glucocorticoid sensitivity and provokes inflammatory signaling. There are at least three plausible mechanistic scenarios. First, chronic stress might dampen the amount of cortisol signal that reaches the monocyte genome. Among people facing lengthy chronic stressors, the diurnal rhythm of cortisol release is often flattened, resulting in lower-than-normal output across the daily cycle (Fries et al., 2005; Lupien et al., 2009; Miller et al., 2007). Under these conditions, monocytes would have lower cortisol exposure, and thus less inhibition of inflammatory activity. Irrespective of cortisol, chronic stress could also downregulate monocyte GR expression, dampening these cells' ability to transduce glucocorticoids' anti-inflammatory signals (Pariante and Miller, 2001). In an earlier study, we found that chronic stress was unrelated to the quantity of GR mRNA expressed by monocytes (Miller et al., 2008). However, there is significant post-transcriptional regulation of GR message, such that only a portion is eventually translated into protein. Thus, to address this issue convincingly, studies of chronic stress and monocyte GR protein expression are needed.

Second, there is considerable functional heterogeneity among monocytes (Auffray et al., 2009; Woollard and Geissmann, 2010). via selective myelopoiesis, chronic stress could mobilize a subpopulation of monocytes with pro-inflammatory and cortisol-resistant tendencies. A recent mouse study found that repeated social defeat caused expansion and mobilization of Ly-6c^{high} cells into peripheral lymphoid tissues (Powell et al., 2013). Ly-6c^{high} cells are immature, pro-inflammatory monocytes, with a functional counterpart in humans identified as CD14+/CD16−. In humans, chronic stress could selectively populate lymphoid organs with these cells, creating an environment marked by glucocorticoid resistance and inflammatory signaling. This possibility has not yet been examined in studies of chronically stressed humans.

Finally, chronic stress could bring about functional alterations that diminish monocytes' capacity to transduce cortisol signals. For example, stress evokes post-translational modifications to proteins comprising GR (Pace et al., 2007). Some of these modifications can engender glucocorticoid resistance (Galliher-Beckley and Cidlowski, 2009). Nevertheless, transcriptional profiling studies published to date (Cole et al., 2011; Miller et al., 2008; O'Donovan et al., 2011) have not simultaneously reported genomic and functional outcomes. Thus, it remains unclear whether bioinformatic indications of glucocorticoid insensitivity among chronically stressed individuals are paralleled by functional indications, e.g., apparent in an *ex vivo* assay system.

In this article we present a multiwave study that builds upon previous research by considering these scenarios. It follows subjects as they grapple with a severe chronic stressor – caring for a family member with glioblastoma multiforme (GBM), an aggressive brain tumor. GBM treatment can be painful and disabling, and most patients die within a year of diagnosis. Even with promising new therapies, two-year survival rates are 27% (Stupp et al., 2005). As a consequence, GBM caregivers face numerous challenges, which can include watching a loved one deteriorate, anticipatory grieving of their death, and marked changes in family interpersonal dynamics. For some families, GBM treatment also poses a significant financial burden, which is compounded if the caregiver must quit work to assist with patient care. These challenges can have implications for health; a recent study showed that in the years following a spouse's cancer diagnosis, caregivers' rates of heart disease and ischemic stroke rose by 13% and 24%, respectively (Ji et al., 2012). Thus, GBM caregivers represent a scientifically and clinically relevant population in which to conduct behavioral immunology research. Here, we draw on them to clarify the linkages among chronic stress, glucocorticoid sensitivity, and inflammatory signaling, focusing specifically on the three mechanistic scenarios outlined above – reduced availability of cortisol or its receptor, selective expansion or mobilization of monocyte subsets, or functional alterations in glucocorticoid signal transduction.

2. Methods

2.1. Sample

Though our group has studied GBM caregivers previously (Miller et al., 2008; Rohleder et al., 2009), the data reported here are from an entirely new sample. There is no data overlap with our past studies. GBM caregivers were recruited from the CNS tumor clinics at the British Columbia Cancer Agency, Vancouver Centre. All caregivers who attended clinic prior to the onset of radiotherapy were approached about participation. Controls were recruited from the broader Vancouver community using advertisements in local media. They had to be without major stressors in their lives during the past year, including divorce, bereavement, unemployment, victimization, significant illness, hospitalization, or care giving responsibilities of their own. The project was approved by the Research Ethics Boards of the University of British Columbia and the British Columbia Cancer Agency, and all subjects gave written consent.

2.2. Assessments

Subjects completed four data-collection sequences. Each consisted of an in-person assessment and three days of ambulatory monitoring. GBM treatment typically involves surgical resection of the tumor, followed by radiotherapy and/or chemotherapy. Caregivers were enrolled after their family member had recovered

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