Brain, Behavior, and Immunity 37 (2014) 78–83

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Short Communication

Divergent gene expression responses to Complicated Grief and Non-complicated Grief

Mary-Frances O'Connor [a,*,1], Christian R. Schultze-Florey [a,b,c,1], Michael R. Irwin [c], Jesusa M.G. Arevalo [d], Steven W. Cole [c,d]

[a] Department of Psychology, University of Arizona, Tucson, AZ 85750, USA
[b] Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany
[c] Cousins Center for Psychoneuroimmunology, Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, CA 90095, USA
[d] Department of Medicine, University of California, Los Angeles, CA 90095, USA

ARTICLE INFO

Article history:
Received 16 September 2013
Received in revised form 6 December 2013
Accepted 20 December 2013
Available online 29 December 2013

Keywords:
Complicated Grief
Bereavement
Widowhood
Gene transcription
gene expression
Immune system
Antiviral
Type I interferon

ABSTRACT

The "widowhood effect" (i.e., morbidity/mortality in recently bereaved spouses) may be related to changes in immune function, but little is known about the impact of bereavement on gene transcription in immune cells. This study examined how Complicated Grief and Non-complicated Grief responses to bereavement differentially affect leukocyte gene expression. Genome-wide transcriptional profiling and bioinformatic analyses were completed on 63 older adults. Thirty-six of them had lost their spouse/partner on average 2 years ago, and 27 were nonbereaved, married controls. Twelve of the bereaved participants met criteria for Complicated Grief. Compared to nonbereaved controls, bereavement (both Complicated Grief and Non-complicated Grief) was associated with upregulated expression of genes involved in general immunologic activation and a selective downregulation of genes involved in B lymphocyte responses. However, Complicated Grief and Non-complicated Grief differed markedly in their expression of Type I interferon-related transcripts, with Non-complicated Grief subjects showing substantial upregulation relative to nonbereaved controls and Complicated Grief subjects showing substantial downregulation. Bereavement significantly modulates immune function gene expression. The magnitude of bereavement-related distress (i.e., Complicated Grief vs. Non-complicated Grief) is linked to differential patterns of transcription factor activation and gene expression involved in innate antiviral responses. These findings provide a molecular framework for understanding the health effects of bereavement, as well as new insights into the particular gene modules that are most sensitive to the individual's psychological response to loss.

1. Introduction

An extensive body of literature shows an increased risk for morbidity and mortality following bereavement (Boyle et al., 2011), and distress-related changes in immune cell function have been hypothesized as key mediator for these effects (Glaser and Kiecolt-Glaser, 2005). A long history of research has identified bereavement-related changes in lymphocyte function and their relative proportions in circulation (Bartrop et al., 1977; Gerra et al., 2003; Irwin et al., 1988). Bereaved widows also show reduced antibody titers to vaccination (Phillips et al., 2006).Physical health effects may be particularly pronounced in bereaved persons with specific inflammatory genetic polymorphisms (Schultze-Florey et al., 2012). Given that bereavement has effects on physical health beyond one year (Martikainen and Valkonen, 1996), immunological alterations may persist long after the death event.

In response to bereavement, the majority of persons cope resiliently with this potentially traumatic event (Bonanno et al., 2002). However, a disorder termed Complicated Grief (CG) affects about 7% of bereaved persons and about 20% of conjugally bereaved (Kersting et al., 2011). CG is characterized by persistent intense grief with ongoing separation distress (Prigerson et al., 2009; Shear et al., 2011). Bereaved individuals who do not have CG may still experience intermittent distress (e.g., sadness), but such Non-complicated Grief (Non-CG) reactions do not significantly impair their interpersonal or emotional functioning.

To clarify how bereavement influences immune system function more broadly, and to determine how CG might differ from Non-CG in its immunologic effects, we assessed leukocyte genome-wide transcriptional profiles in relationship to empirically-validated diagnostic criteria for CG (Prigerson et al., 1995a; Shear et al., 2011). Previous social genomics studies have linked

* Corresponding author. Address: Department of Psychology, University of Arizona, 1503 E. University Boulevard, Room 430, Tucson, AZ 85721, USA. Tel.: +1 520 621 2173; fax: +1 520 621 9306.
E-mail address: mfoconnor@email.arizona.edu (M.-F. O'Connor).
[1] Authors contributed equally.

0889-1591/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbi.2013.12.017

other types of social adversity (e.g., isolation, stress, low socioeconomic status (SES)) with a Conserved Transcriptional Response to Adversity (CTRA) characterized by downregulation of antiviral genes and upregulation of inflammation-related genes (Irwin and Cole, 2011). Similar effects were seen in caregiving spouses of brain cancer patients (Miller et al., 2008).

In the present preliminary study we sought to determine: (1) to what extent one of the most intense social adversities (i.e., bereavement) can modulate leucocyte gene expression, and (2) whether these dynamics are more pronounced for those reacting to bereavement with greater distress (i.e., with CG). Consistent with patterns of gene transcriptional responses to other major life adversities, we hypothesized that bereavement would show the CTRA pattern of increased expression of immune activation-related genes (CG more than Non-CG), and decreased expression of antiviral genes (CG more than Non-CG). Monocytes and dendritic cells are implicated as primary mediators of the CTRA (Cole et al., 2011). However, because of altered function of natural killer (NK) cells in bereavement (Gerra et al., 2003; Irwin et al., 1988) we hypothesized that NK cell-expressed genes might also be affected (CG more than Non-CG).

2. Methods and materials

2.1. Participants

We recruited 63 older adults (age 61–83) from the Los Angeles community through advertisement at senior centers and direct mailing to age-appropriate citizens. Interested participants were then categorized as two groups. Thirty-six participants had experienced the death of their spouse or partner on average in the past 2 years (mean: 23.56 months, SD 16.10), with 12 of the subjects meeting criteria for CG (Prigerson et al., 1995b). The other 27 participants were nonbereaved married/partnered control subjects who had not lost a first-degree relative or spouse within the prior 36 months. The UCLA Human Research Protection Program approved the study and all participants gave written informed consent after complete description of the study. As described in our previous report on genetic effects (Schultze-Florey et al., 2012), exclusion criteria included: (a) presence of a current major psychiatric disorder (e.g., major depressive disorder, post-traumatic stress disorder, alcohol dependence) as assessed with the Structured Clinical Interview for DSM-IV (SCID-I; Spitzer et al., 1994); (b) psychotropic medication use initiated after the death event; (c) immunosuppressive medication (d) major medical illnesses (e.g., cancer); (e) current smokers.

2.2. Psychological measures

All participants received the Perceived Stress Scale (PSS) (Cohen et al., 1983) ($\alpha = 0.81$), the UCLA Loneliness Scale (Russell, 1996) ($\alpha = 0.89$), and the revised Social Readjustment Rating Scale (SRRS-R) (Hobson and Delunas, 2001).

Bereaved participants were given the Inventory of Complicated Grief (ICG) (Prigerson et al., 1995b), to assess symptoms and behavior that define CG. Consistent with prior studies, the cut-off for CG was >30 (Shear et al., 2005) ($\alpha = 0.90$). The Impact of Events Scale (IES) (Horowitz et al., 1979) ($\alpha = 0.85$) and the Yearning in Situations of Loss (YSL) Scale (O'Connor and Sussman, 2014) ($\alpha = 0.94$) were also administered to all bereaved participants.

2.3. Gene expression profiling

Five million peripheral blood mononuclear cells (PBMCs) were isolated and total RNA was extracted (RNeasy; Qiagen, Valencia, CA), tested for suitable mass (NanodropND1000; Thermo Scientific, Rockford, IL) and integrity (Bioanalyzer 2100; Agilent, Santa Clara, CA), and subjected to genome-wide transcriptional profiling using Illumina Human HT-12 v3 Expression BeadChips (Illumina Inc., San Diego, CA), following the manufacturer's standard protocol in the UCLA Southern California Genotyping Consortium Core Laboratory, described previously (Cole et al., 2007, 2010). Gene expression values were quantile normalized (Bolstad et al., 2003) and transformed to $\log_2$ for genome-wide general linear model analyses that controlled for age, sex, race (Caucasian vs. non-Caucasian), education (years of schooling), current employment status, body mass index (BMI), and alcohol consumption. Differentially expressed genes were identified by adjusted parameter estimates exceeding a 1.25-fold difference between groups. No statistical testing was applied at the level of individual genes because this study had no single-gene hypotheses and this study was not designed to detect statistically significant associations between single gene transcripts and bereavement status. Differentially expressed genes were identified only to serve as intermediate inputs into higher-order bioinformatics analyses that maintain their own false positive statistical control in analyses of Gene Ontology (GO) functional characteristics, transcription control pathways, and originating cell types, as previously detailed (Fredrickson et al., 2013).

Functional characteristics of differentially expressed genes were identified by GOstat (http://gostat.wehi.edu.au/) Gene Ontology (GO) analysis with False Discovery Rate-adjusted *p*-values (Beissbarth and Speed, 2004). To assess *a priori* hypotheses regarding specific transcription control pathways that might contribute to observed effects, we used TELiS (http://www.telis.ucla.edu/) bioinformatic analysis (Cole et al., 2005) of transcription factor-binding motifs in promoters of differentially expressed genes (NF-κB motifs assessed by TRANSFAC matrix V$CREL_01, Type I interferon response factors (IRFs) V$ISRE_01 and IRF1_01, cAMP response element-binding protein (CREB) V$CREB_02, and GATA-binding protein 1 (*GATA1) V$GATA1_04).* To identify specific leukocyte subsets predominately mediating the observed differences in gene expression, Transcript Origin Analysis (TOA) (Cole et al., 2011) was carried out. TELiS and TOA analyses were carried out in an *a priori* hypothesis testing format (i.e., only specifically hypothesized effects were subject to statistical testing and reporting in primary results; comprehensive exploratory/discovery findings are reported separately as such in supplemental data files).

2.4. Statistical analysis

SPSS 19 (SPSS, Chicago, IL, USA) was used for statistical analyses. ANOVA or chi-square analyses and t-tests were used for planned *post hoc* group comparisons. To analyze relationships while controlling for demographic, medical, or biobehavioral confounds regression analyses were used. Significance was defined as *p*-values <0.05.

3. Results

3.1. Demographic and psychological characteristics

Demographic characteristics of the three groups are shown in Table 1. ANOVA analysis and *post hoc t*-test showed that the Non-CG group had been married fewer years than the Control group ($t = 2.35$, $p = 0.02$). Importantly, unexpected deaths (defined in the present study as knowing that the spouse would die for less than one week) were not significantly different between CG and Non-CG[2] (Currier et al., 2006).

[2] Debate in the literature exists as to whether unexpectedness causes greater bereavement distress and whether it is predictive of CG (for a review, see Currier et al., 2006.)