Brain, Behavior, and Immunity 26 (2012) 1066–1071

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

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

When grief makes you sick: Bereavement induced systemic inflammation is a question of genotype

Christian R. Schultze-Florey [a,b], Otoniel Martínez-Maza [c], Larry Magpantay [c], Elizabeth Crabb Breen [a], Michael R. Irwin [a], Harald Gündel [d], Mary-Frances O'Connor [e,*]

[a] Cousins Center for Psychoneuroimmunology, Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, CA 90095, USA
[b] Hannover Medical School, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany
[c] Departments of Obstetrics and Gynecology and Microbiology, Immunology, and Molecular Genetics, David Geffen School of Medicine, and Department of Epidemiology, UCLA School of Public Health, University of California, Los Angeles, CA 90025, USA
[d] Universitätsklinik für Psychosomatische Medizin und Psychotherapie, Universitätsklinikum Ulm, Universität Ulm, Am Hochsträss 8, D-89081 Ulm, Germany
[e] Department of Psychology, University of Arizona, Tucson, AZ 85750, USA

ARTICLE INFO

Article history:
Received 12 April 2012
Received in revised form 17 June 2012
Accepted 17 June 2012
Available online 23 June 2012

Keywords:
Aging
Bereavement
Cytokines
Gene
Gene by environment
Grief
IL-1
IL-6, TNF-a
Inflammation

ABSTRACT

Although bereavement is associated with increased morbidity and mortality in the surviving spouse, some widow(er)s remain healthy. Genetic variability in expression of inflammatory markers in response to stress may be the key to this observation. The present study compares bereaved *vs.* married/partnered older adults, investigating the impact of bereavement status, pro-inflammatory cytokine single nucleotide polymorphisms (SNPs) on circulating markers of inflammation and hypothesizing a gene by environment (GxE) effect. The study sample included 64 older adults, of which 36 were widow(er)s. Circulating levels of inflammatory markers IL-6, IL-1RA and sTNFRII were measured. Participants were genotyped for SNPs in the *IL-6* gene (*IL-6* −174 and −572), the *IL-1β* gene (*IL-1β* −511), and *TNF-α* gene (*TNF-α* −308). Grief severity was assessed with the Inventory of Complicated Grief. Bereaved participants had higher circulating levels of IL-1RA and IL-6. This increase could not be explained by pro-inflammatory genotype frequency differences, or Complicated Grief diagnosis. However, a GxE effect with the *IL-6* −174 SNP moderated individual vulnerability to higher circulating levels of inflammation resulting from bereavement exposure. These results suggest a possible mechanism for the increase in morbidity and mortality in the surviving spouse. Genetic variability interacts with an environmental stressor, leading to increased inflammatory markers in genetically susceptible subjects only. For these patients, clinical interventions for bereavement-related stressor reduction might be crucial for overall health.

1. Introduction

The death of a spouse is one of the most distressing life events, and is associated with an increase in morbidity and mortality risk, independent of a host of covariates (Boyle et al., 2011). Stressful life events are linked to dysregulation of the immune system and increased cellular inflammatory signaling (Irwin and Cole, 2011). Because it is our long-term goal to determine the mechanism linking bereavement and morbidity/mortality, and because substantial evidence shows that increases in inflammatory markers such as interleukin (IL)-6 are associated with mortality risk (Ershler and Keller, 2000), we investigated whether responses to spousal bereavement are impacted by markers of inflammation.

Chronic stress as a result of caregiving (i.e., those taking care of a severely ill family member) or social isolation is associated with the up-regulated gene expression and production of systemic markers of inflammation (Kiecolt-Glaser et al., 2003; McDade et al., 2006), especially of IL-6, IL-1 and TNF-alpha (Dantzer, 2001). Further data indicate genetic variability in the expression of inflammatory markers in response to stress (Cole et al., 2010, 2011). For example, the presence of the guanine/cytosine (G/C) single nucleotide polymorphism (SNP) in the promoter of the *IL-6* gene (*IL-6*) −174 bp upstream of the transcription start site affects the binding of a β-adrenergic-sensitive transcription factor, GATA-1 (Cole et al., 2010). Following an *in vitro* β-adrenergic stimulus, the *IL-6* −174G SNP leads to increased IL-6 production, while the *IL-6* −174C SNP does not. *In vivo*, an association between depression and increased mortality risk was found only in *IL-6* −174GG homozygous patients (Cole et al., 2010), but levels of inflammatory markers were not characterized. Thus, it is not known whether genetic variability in cytokine gene polymorphisms alters the

* 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).

0889-1591/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbi.2012.06.009

influence of life stress on the production of inflammatory markers in humans.

Given evidence that some widow(er)s show an increase in morbidity and mortality, but others remain healthy and thus appear to be protected from this "widowhood effect" (Buckley et al., 2011), the present study hypothesizes that bereavement may be a socio-environmental stress that interacts with pro-inflammatory genetic variation to produce modulated levels of inflammation. This gene by environment (GxE) interaction predicts that among widow(er)s, those with genotypes that are associated with higher expression of inflammatory markers would exhibit greater systemic signs of inflammation, e.g. higher circulating levels of inflammatory markers. Conversely, widow(er)s with low pro-inflammatory genotypes are hypothesized to have similar circulating levels as non-bereaved individuals.

The work reported here compares bereaved and healthy married/partnered older adults, first investigating the impact of bereavement status on circulating levels of inflammatory markers IL-6, IL-1RA as a marker of IL-1 activity (Arend et al., 1998) and soluble tumor necrosis factor receptor II (sTNFRII) as a surrogate marker of TNF-α levels (Diez-Ruiz et al., 1995; Schuld et al., 1999). Second, corresponding to our circulating measures, we investigated the impact of pro-inflammatory cytokine SNPs in the *IL-6*, *IL-1β*, and tumor necrosis factor alpha *(TNF-α* genes on the mentioned inflammatory markers and a possible interaction with bereavement status. Given evidence of *in vitro* data for the GxE pathway for the *IL-6* −174 SNP, we primarily focused on the interaction between bereavement stress and this polymorphism.

2. Methods and materials

2.1. Participants

A total of 64 older adults (age 61–83) were recruited from the Los Angeles community. Thirty-six were widowed; on average they had experienced the death of their spouse or partner in the past 2 years (mean: 23.75 months; range 2–69 months). Widowhood was defined as the death of a co-habiting life partner of the same generation, although not necessarily legally married (the majority were married). The other 28 participants were non-bereaved control subjects who were married/partnered and had not lost a first-degree relative or spouse in the prior 36 months.

The sample of 64 participants was mainly of Caucasian descent (77%). Other ethnicities included African-American (13%), Hispanic (6%), Asian (2%), Pacific Islander (1%) and other (1%). For statistical analyses we created a dichotomous variable (Caucasian *vs.* Non-Caucasian).

To assess the level of stress resulting from life events among all participants, we used the revised Social Readjustment Rating Scale (SRRS-R) (Hobson and Delunas, 2001). The SRRS-R covers 51 life events that may have occurred in the past 12 months. In addition, perceived stress was assessed with the Perceived Stress Scale (PSS), a 14-item measure of the degree to which respondents appraise stressful situations that occurred during the past month (Cohen et al., 1983). In our study internal consistency was $\alpha = .82$.

Exclusion criteria included: (a) presence of current major psychiatric disorder (e.g., Major Depressive Disorder, alcohol or substance dependence) as assessed with the DSM-IV SCID-I (Spitzer et al., 1994); (b) use of psychotropic medications initiated since the death event; (c) immunosuppressive medication (d) major medical illnesses (e.g., cancer); (e) current smokers (due to potential confounding effects on markers of inflammation) (O'Connor et al., 2009); (f) participants with IL-6 levels >2 standard deviations above the mean, if there was self-reported illness in the past two weeks.

Bereaved participants were given the Inventory of Complicated Grief (ICG) (Prigerson et al., 1995). Consistent with prior studies we used an ICG total score of 30 or higher for diagnosis of Complicated Grief (Shear et al., 2005). Internal consistency was $\alpha = .90$.

The Institutional Review Board of UCLA approved the study and after complete description of the study all participants gave written informed consent.

2.2. Levels of circulating markers of inflammation

EDTA plasma was collected and stored at −80 °C until all samples were obtained. IL-6 was measured using Quantikine High Sensitivity Immunoassay kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. IL-1RA and sTNFRII were measured with Quantikine Immunoassay kits (R&D Systems).

All samples were assayed in duplicate, and an internal quality control sample was included on every assay; the interassay and intraassay coefficients of variation were less than 8% and 5%, respectively. None of the circulating inflammatory markers were below the limit of detection. Personnel who performed immunoassays were blinded to the identity and diagnostic status of study participants.

2.3. DNA extraction and genotyping

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples using Ficoll separation and stored at −80 °C until processing. Genomic DNA was isolated with Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) followed by determination of concentration and purity. SNPs in the genes of interest were determined with TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA). Corresponding to our circulating measures we investigated SNPs that have been identified as influencing quantitative gene expression levels: *IL-6* −174 (Fishman et al., 1998; Olomolaiye et al., 1998) and −572 (Terry et al., 2000); *IL-1β* −511 (di Giovine et al., 1992); and *TNF-α* −308 (Wilson et al., 1992).

For the *IL-6* −174 G/C and *IL-6* −572 G/C polymorphisms we used Custom TaqMan® SNP Genotyping Assays; primer sequences are shown in Table 1. Custom assays were run with control DNA of known genotype, for confirmation of assay quality control. The other two SNPs (*IL-1β* −511 A/G, *TNF-α* −308 G/A) were genotyped using commercially available TaqMan® SNP Genotyping Assays. These assays were used according to the manufacturer's instructions. Genotyping for all samples was done in duplicate (concordance >.99) with call rates of all SNPs being >.95. Investigators who performed SNP analyses were blinded to the identity and diagnostic status of the study participants.

2.4. Statistical Analysis

Statistical analyses were carried out using SPSS 19 (SPSS, Chicago, IL, USA). Group comparisons were assessed by ANOVAs,

Table 1
Primer and probe sequences used for genotyping of *IL-6* −174 and *IL-6* −572 SNP.

Primer name	*Sequence 5′–3′*
IL-6 −174 forward	GACGACCTAAGCTGCACTTTTC
IL-6 −174 reverse	GGGCTGATTGGAAACCTTATTAAGATTG
IL-6 −572 forward	GCCTTGAAGTAACTGCACGAAATT
IL-6 −572 reverse	CCAGTCATCTGAGTTCTTCTGTGTT
Probe name	*Sequence 5′–3′*
IL-6 −174C VIC	CTTTAGCATGGCAAGAC
IL-6 −174G FAM	CTTTAGCATCGCAAGAC
IL-6 −572G VIC	TACAACAGCCCCTCACAG
IL-6 −572C FAM	AACAGCCGCTCACAG

A = adenine, C = cytosine, G = guanine, T = thymine; VIC = VIC®; FAM = 6-carboxyfluorescein.