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Telomere shortening and immune activity in war veterans with posttraumatic stress disorder

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ABSTRACT

Background: There is increasing evidence that chronic stress accelerates telomere erosion in leukocytes/peripheral blood mononuclear cells (PBMCs). However, functional changes associated with telomere shortening are poorly understood. We hypothesized that war veterans with PTSD would have shorter telomeres in PBMCs and that these cells might exhibit changes in measures of immune reactivity such as proliferation, cytokine production and expression of regulators of immune responses.
Methods: We measured relative telomere length and basal telomerase activity in PBMCs of 62 individuals (PTSD patients (N = 30); age-matched healthy controls (N = 17), elderly volunteers (N = 15)). In parallel, we have assessed proliferation of activated T cells, interferon (IFN)-γ, interleukin (IL)-2, IL-4, tumor necrosis factor (TNF)-α and IL-6 cytokine production and expression of programmed death 1 (PD-1) receptor and its ligand PD-L1 on activated T cells.
Results: Middle-aged war veterans with current PTSD had shorter PBMC telomere length than their age-matched healthy controls while the elderly had the shortest telomeres. There was no difference in telomerase activity between PTSD patients and healthy controls while telomerase activity was significantly lower in the elderly. While the elderly group exhibited robust changes in immune activity such as increased production of proinflammatory cytokines (TNF-α, IL-6) and reduced proliferation of all T cells, the PTSD group showed reduced proliferative response of $CD8^+$ T cells to high concentrations of mitogen and reduced spontaneous production of IL-2 and IFN-γ.
Conclusions: This study adds to the accumulating evidence that psychological trauma and chronic stress are associated with accelerated telomere attrition. However, changes in immune function associated with stress-related telomere shortening are not well understood. Although much less pronounced in PTSD patients than in elderly persons, reduced proliferative responses of T cells accompanied by shorter telomeres might be a sign of early immunosenescence. Together with reduced production of Th1 cytokines, observed immune changes may contribute to health risks associated with PTSD.

1. Introduction

Telomeres are repeating hexameric sequences of DNA that are found at the ends of linear chromosomes in association with a complex of proteins; their role is to maintain chromosome integrity and stability (Blackburn, 2001). Telomeric DNA is lost due to the incomplete terminal synthesis of the lagging DNA strand during cell division (Newlon, 1988). The rate of telomere loss is slowed by the enzyme telomerase, an RNA-dependent DNA polymerase that synthesizes telomeric repeats and thus maintains telomeres during cell replication (Greider and Blackburn, 1985). Telomerase is expressed in cells of the germinal line, stem cells and some leukocytes but repressed in normal somatic cells (Gomez et al., 2012), thus telomeres progressively shorten with aging (Counter et al., 1992). In addition to replicative DNA loss, telomere shortening can also result from exposure to genotoxic stressors such as reactive oxygen species and UV radiation (Morgan, 2013). When telomeres reach a critically short length, they are recognized as double-stranded DNA breaks that activate the p53 tumor suppressor protein resulting in cell senescence or apoptosis (Collado et al. 2007).

Abbreviations: BDI, Beck Depression Inventory; BMI, Body Mass Index; CAPS, Clinician Administered PTSD Scale; LPS, lipopolysaccharide; LTL, leukocyte telomere length; M.I.N.I., Mini International Neuropsychiatric Interview; PBMCs, peripheral blood mononuclear cells; PHA, phytohemagglutinin; *PMA*, *phorbol-12-myristate-13-acetate*; PD-1, programmed death 1; PTSD, posttraumatic stress disorder; STAI, State-Trait Anxiety Inventory.

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Inverse correlation between telomere length in various human tissues and age-related diseases is well described (von Zglinicki and Martin-Ruiz, 2005). Although telomerase is active in some (mostly activated) leukocytes, telomeres in leukocytes shorten with age and leukocyte telomere length (LTL) is considered a reliable marker of biological age (Müezzinler et al. 2013) that predicts the onset of age-related diseases (Epel et al., 2009; Fitzpatrick et al., 2007; Panossian et al., 2003) and mortality (Cawthon et al., 2003; Kimura et al., 2008).

The first evidence that psychological stress may impact telomere maintenance came from a study which described shorter telomere length of peripheral blood mononuclear cells (PBMCs) in mothers of chronically ill children (Epel et al., 2004). PBMCs include two major subpopulations of leukocytes, namely lymphocytes and monocytes, but no granulocytes. In recent years, telomere shortening has been implicated in various psychiatric conditions such as mood disorders (Simon et al., 2006), posttraumatic stress disorder (PTSD) (O'Donovan et al., 2011), and in individuals that have experienced childhood trauma (Shalev et al., 2013). Most of these studies measured telomere length in leukocytes/PBMCs of the participants but lacked parallel assessment of functional reactivity of these cells with shortened telomeres. To our knowledge only one study (Damjanovic et al., 2007) assessed functional consequences of telomere shortening of PBMCs in human subjects undergoing chronic stress and found that telomere erosion was associated with declining immune function in caregivers of Alzheimer's disease patients.

With this in mind, the aim of the current study was to measure telomere length in PBMCs of Croatian war veterans with PTSD and to assess immune reactivity of these cells in the context of immunosenescence (Aw et al., 2007), i.e., age-dependent decline of immune function. For this purpose, the study included a comparison group of elderly persons (age > 80).

PTSD is a trauma-related disorder that may develop after exposure to one or more traumatic events (American Psychiatric Association, 2013) and is associated with various biological and behavioral changes that constitute a higher risk for developing somatic illness (Barrett et al., 2002; Boscarino, 2004) and higher mortality (Xue et al., 2012). Studies of the immune system in PTSD have produced conflicting results although there is evidence for increased peripheral inflammation manifested by increased levels of pro-inflammatory cytokines (IL-1β, IL-6 and tumor necrosis factor (TNF)-α) (Baker et al., 2012; Pace and Heim, 2011). Immune dysregulation in PTSD and other chronic stress models seems to resemble changes induced by biological aging (Alvarez-Rodríguez et al., 2012; Andrews and Neises, 2012).

The present study measured relative telomere length and basal telomerase activity in PBMCs of patients with PTSD, age-matched healthy controls and a group of elderly volunteers. In parallel, we assessed proliferation of activated T cells, and cytokine production by unstimulated or stimulated monocytes and T cells. In addition, we measured expression of programmed death 1 (PD-1) receptor and its ligand PD-L1, negative regulators of immune responses (Keir et al., 2008), on activated T cells. PD-1 is a member of the CD28/CTLA-4 family of T cell regulators, expressed on the surface of activated T cells, B cells and myeloid cells (Agata et al., 1996; Ishida et al., 1992). The main ligand for PD-1, PD-L1 induces a co-inhibitory signal in activated T-cells and promotes T-cell apoptosis, anergy and functional exhaustion (Shi et al., 2013). Thus, PD-1 and PD-L1 interaction is important for co-inhibition during the T-cell initiation of an immune response, and the importance of this interaction is highlighted by the autoimmune phenotype of PD-1 knockout mice (Nishimura et al., 1999, 2001).

2. Materials and methods

2.1. Participants

PTSD patients (N = 30) were Croatian male combat veterans, recruited among outpatients at the "Dr. Josip Benčević" General Hospital, Slavonski Brod, Croatia. Healthy controls (N = 17) were men of similar age from the same area. The elderly control group consisted of 15 persons (male N = 2, female N = 13) aged 80 or older.

War veterans met diagnostic criteria for PTSD based on the ICD-10 (WHO, 1992). They were severely traumatized during the war in Croatia (1991–1995) and have undergone several forms of psychiatric treatment since the war ended. For purposes of this study assessments of war veterans and healthy controls were made by structured interviews, the Croatian versions of the Mini International Neuropsychiatric Interview (M.I.N.I.; (Sheehan et al., 1998)) and the Clinician Administered PTSD Scale (CAPS) (Blake et al., 1995). Assessments were made by psychiatrists who had been trained in the administration of the specific instruments. The participants from these groups also underwent a physical examination in order to assess for symptoms or signs of acute or chronic physical illnesses.

War veterans and healthy controls completed a questionnaire which included: basic demographic characteristics, weight, height, alcohol consumption, smoking, physical activity, presence of acute or chronic physical illness, and medication use. They were then asked to complete rating scales for depression and anxiety symptoms. Depression symptoms were assessed with the Beck Depression Inventory (BDI) (Beck et al. 1988) and current anxiety level (state and trait anxiety) was determined by the Spielberger State-Trait Anxiety Inventory (STAI) (Spielberger et al. 1970).

Participants from the control group were excluded from the study if they had a history of acute psychosis, dementia, schizophrenia, mood disorders, or personality disorders. The exclusion criteria for war veterans as well as healthy controls included: substance abuse, symptoms or signs of acute or chronic physical illnesses, including infectious, allergic or endocrine disorders.

A blood draw was performed after the initial psychiatric interview and physical examination but before the structured psychiatric interview and completion of questionnaire and rating scales to minimize the influence of acute stress on the results.

A group of elderly controls was recruited from an aged care nursing home in„ Slavonski Brod". They volunteered to participate in the study. Data obtained from this group served as a biological control for telomere shortening and age-related changes in immune reactivity. The only inclusion criteria were age > 80 and no history or current neoplastic disorders or autoimmune diseases.

The study was approved by the Ethics Committee of the "Dr. Josip Benčević" General Hospital, Slavonski Brod, Croatia, and written informed consent was obtained from all subjects.

2.2. Blood sampling

Fasting whole blood of the participants was collected by venipuncture in sodium heparin treated tubes (BD Biosciences, Heidelberg, Germany), between 7 and 9 AM. Peripheral blood mononuclear cells (PBMCs) were isolated on a Ficoll-Paque™ gradient (GE Healthcare Life Sciences, Uppsala, Sweden). Upon separation, mononuclear cells were washed, resuspended in freezing medium (10% FCS, 10% DMSO, 80% RPMI 1640) and transferred within a freezing container (Sigma-Aldrich, St. Louis, USA) to −80 °C overnight, then stored in liquid nitrogen until further processing.

2.3. Measurement of telomere length and telomerase activity

Genomic DNA was isolated from PBMCs by standard phenol–chloroform extraction (Sambrook and Russell, 2006). Concentration and purity of the genomic DNA were determined by 260/280 UV spectrophotometry. Relative telomere length was measured by multiplex quantitative PCR method as previously described (Cawthon, 2009). Briefly, this method describes the relative telomere length as the ratio (T/S) of the telomere repeat copy number (T) to a single copy gene (S). This ratio is measured relative to a standard DNA which in this case was a pooled DNA sample of several healthy