Brain, Behavior, and Immunity xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides

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ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form 2 May 2014
Accepted 12 May 2014
Available online xxxx

Keywords:
Depression
Suicide
Microglia
Perivascular macrophages
Anterior cingulate cortex
Neuroinflammation
Human

ABSTRACT

Despite increasing evidence supporting the neuroinflammatory theory of depression, little is known about cerebral macrophages in individuals suffering from major depression. In the present study, we investigated the morphology and distribution of cells immunostained for the macrophage-specific marker ionized calcium binding adaptor molecule 1 (IBA1) in the dorsal anterior cingulate cortex (dACC) white matter of middle-aged depressed suicides and matched non-psychiatric controls. This region is known for its implication in mood disorders, and its white matter compartment was previously found to display hypertrophic astrocytes in depressed suicides. Distributions of IBA1-immunoreactive (IBA-IR) microglial phenotypes were assessed using stereology and cell morphometry, and blood vessels were characterized as being intimately associated with either a high or a low density of IBA1-IR amoeboid-like cells. Total densities of IBA1-IR microglia did not differ between depressed suicides and controls. However, a finer analysis examining relative proportions of microglial phenotypes revealed that the ratio of primed over ramified (''resting'') microglia was significantly increased in depressed suicides. Strikingly, the proportion of blood vessels surrounded by a high density of macrophages was more than twice higher in depressed suicides than in controls, and this difference was strongly significant. Consistent with these observations, gene expression of IBA1 and MCP-1, a chemokine involved in the recruitment of circulating monocytes, was significantly upregulated in depressed suicides. Furthermore, mRNA for CD45, a marker enriched in perivascular macrophages, was also significantly increased in samples from depressed suicides. An increase compared to controls was also observed in the proportion of blood vessels surrounded by a high density of CD45-IR cells, but this difference did not reach significance. These histological and molecular data suggest the recruitment of monocytes in dACC white matter of depressed suicides, although it cannot be excluded that other types of macrophages (including microglia) account for the observed accumulation of macrophages closely associated with blood vessels. Altogether, these findings suggest that the previously reported depression- and suicide-associated increases in circulating pro-inflammatory cytokines may be associated with low-grade cerebral neuroinflammation involving the recruitment of circulating monocytes.

1. Introduction

Major depressive disorder (MDD) affects approximately 350 million people worldwide and is ranked as one of the main causes of disability (World Health Organization [WHO], 2012). This severe condition, characterized by depressed mood and/or loss of interest, too often results in suicide completion. Suicide ranks among the top ten causes of death for individuals of all ages and is the leading cause of death in most developed countries for subjects younger than 35 years (WHO, 2006). Psychological autopsy studies indicate that at least 50% of all adult suicides have had a previous diagnosis of depression (Kim et al., 2003). Furthermore, up to 15% of individuals with a lifetime diagnosis of MDD admit having attempted suicide at some point in their lives (Chen and Dilsaver, 1996). This serious societal problem has given rise to an increasingly

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http://dx.doi.org/10.1016/j.bbi.2014.05.007
0889-1591/

multidisciplinary research field aimed at understanding the biological causes underlying depression and suicide. The inflammatory theory of depression stands as one of the main hypotheses having emerged from this research (Dantzer et al., 2008; Miller et al., 2009). It has at its roots a number of independent clinical studies showing that the expression of some peripheral inflammatory markers is increased in depressed patients (Dowlati et al., 2010). In fact, increased expression of circulating pro-inflammatory cytokines has even been proposed as a biomarker of depression (Lichtblau et al., 2013; Maes et al., 2009). Further supporting this hypothesis, the peripheral administration of pro-inflammatory cytokines such as IFN-α for the treatment of hepatitis C leads to depressive symptoms in about half of the patients (Capuron and Miller, 2004; Malaguarnera et al., 1998). This treatment was also reported to activate the dorsal anterior cingulate cortex (dACC) (Capuron et al., 2005), a region that has been shown to display reduced volume and activity in magnetic resonance imaging studies of MDD patients (Chana et al., 2003; Ebert and Ebmeier, 1996) and associated with behavioral response to peripheral inflammation (Miller et al., 2013). These data strongly suggest that circulating pro-inflammatory cytokines may affect mood states through a functional alteration of limbic brain circuits. Further indication that immune activation may have a significant influence on mood comes from the increased incidence of depression in patients suffering from chronic inflammatory illnesses such as coronary artery disease (Frasure-Smith and Lesperance, 2006). Animal studies have also supported this hypothesis, namely by providing strong evidence of a positive correlation between increased circulating pro-inflammatory cytokines and depressive-like behaviors (Goshen et al., 2008; Merali et al., 2003). Despite these converging lines of evidence, less than a handful of studies have examined the expression of cerebral cytokines in individuals having suffered from depression (Dean, 2011; Pandey et al., 2012; Tonelli et al., 2008).

At the cellular echelon, central immune responses are mainly modulated by microglia, but also by astrocytes, which generally play inflammatory and anti-inflammatory roles, respectively (McNally et al., 2008). Perivascular macrophages, which share many features with microglia, are also implicated by mediating the brain's physiological responses to circulating pro-inflammatory cytokines (Serrats et al., 2010). In a murine model of social stress, Wohleb and colleagues recently described that social defeat is accompanied by the priming of monocytes and the recruitment of peripheral macrophages into cerebral perivascular space. This trafficking of myeloid cells in the brain coincides with the activation of resident microglia, the production of pro-inflammatory cytokines and the appearance of anxiety-like behaviours (Wohleb et al., 2012, Wohleb et al, 2013). There currently exists a knowledge gap with regards to these cells in regions implicated in depression and suicide. In fact, little is known about macrophages in the human brain (Guillemin and Brew, 2004), let alone in mood disorders. Interestingly, Steiner and colleagues have reported immunohistological evidence of increased cerebral gray matter HLA-DR-immunoreactive microglial densities in suicide victims, irrespective of psychiatric diagnosis (Steiner et al., 2008). However, the distribution and morphological phenotypes of white matter macrophages remains to be investigated, particularly in the dACC, a region that has been repeatedly implicated in mood disorders and in the behavioral response to inflammation (Capuron et al., 2005; Haroon et al., 2014; Miller et al., 2013). Furthermore, white matter astrocytes in the dACC of depressed suicides display a hypertrophic phenotype suggestive of mild astrogliosis (Torres-Platas et al., 2011). The aim of the present study was to examine the distribution and morphology of macrophages in well-characterized dACC white matter samples from depressed suicides and matched psychiatrically healthy controls.

2. Materials and methods

2.1. Brain samples

This study was approved by the Douglas Hospital Research Ethics Board, and informed consent from next-of-kin was obtained for each subject. Postmortem brain samples from depressed suicides and matched sudden-death controls were provided by the Suicide section of the Douglas-Bell Canada Brain Bank. All psychiatric subjects committed suicide in the context of a major depressive episode, and controls died suddenly without psychiatric, neurological or inflammatory illnesses (Table 1). Seven controls died from sudden cardiac arrest, and one of the depressed suicides overdosed from a cocktail of drugs that included diphenhydramine, an antihistaminergic compound. Fresh-frozen samples were obtained for messenger RNA (mRNA) experiments (16 cases and 14 controls) and fixed samples for the anatomical experiments (14 cases and 8 controls). For each individual, the cause of death was ascertained by the Quebec Coroner's office, and psychological autopsies were performed by proxy-based interviews, as described previously (Dumais et al., 2005). In brief, a trained interviewer conducted the *Structured Clinical Interview for DSM-IV* Psychiatric Disorders (SCID-I) with one or more informants of the deceased, after which a blind panel of clinicians reviewed SCID-I assessments, case reports, coroner's notes and medical records to obtain consensus psychiatric diagnosis. Groups were matched according to age, tissue pH, refrigeration delay, and postmortem interval. All tissue samples were dissected from the dACC adjacent to the dorsal part of the genu of the corpus callosum (Brodmann area 24 [BA24]) (Gittins and Harrison, 2004; Vogt et al., 1995), as described previously (Hercher et al., 2009).

2.2. IBA1 and CD45 immunohistochemistry

Fixed samples were processed as previously described (Torres-Platas et al., 2014). In brief, tissue blocks were cut into 50 μm-thick serial coronal sections and every 12th section was processed for immunohistochemistry (IHC). Antigen retrieval was performed with proteinase K (20 μg/ml) for ionized calcium-binding adapter molecule (IBA1)-IHC and citrate buffer for cluster of differentiation (CD) 45 IHC, followed by an incubation in 3% H_2O_2. Sections were then pre-incubated respectively in 2% normal goat and horse serum for 24 h before being transferred for 48 h in the same solution containing polyclonal rabbit anti-IBA1 (1:1000; WAKO Chemicals USA, Inc., Richmond, VA, USA) or overnight with monoclonal mouse anti-CD45 Leucocyte Common Antigen (1:100; Clones 2B11 & PD7/26; Dako Canada Inc. Burlington, ON, Canada). This was followed by 1 h incubation in biotinylated goat anti-rabbit and anti-mouse antibody, respectively (1:1000; Vector Laboratories Inc., Burlington, ON, Canada). Labeling was revealed with a diaminobenzidine kit (Vector Laboratories Inc., Burlington, ON, Canada) and samples were counter-stained with cresyl violet. All slides were coded and analyzed by an experimenter blind to diagnosis.

2.3. Quantitative assessment of microglial phenotypes

In a recent study, we performed a morphometric characterization of microglial cells in human dACC (Torres-Platas et al., 2014), and generated quantitative parameters allowing us to accurately identify four main phenotypes, based strictly on morphology: ramified, primed, reactive and amoeboid (Fig. 1). IBA1-immunoreactive (IBA1-IR) microglial phenotypes were assessed as followed: Ramified microglia were characterized by a cell body ranging between 2.5 and 4.9 μm in length in its shortest

Please cite this article in press as: Torres-Platas, S.G., et al. Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides. Brain Behav. Immun. (2014), http://dx.doi.org/10.1016/j.bbi.2014.05.007