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Monocyte activation detected prior to a diagnosis of schizophrenia in the US Military New Onset Psychosis Project (MNOPP)

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ABSTRACT

Low-grade inflammation is present in some cases of schizophrenia, particularly in the early stages of this disorder. The inflammation source is not known but may be the result of dysbiotic processes occurring in the gut. We examined peripheral biomarkers of bacterial translocation, soluble CD14 (sCD14) and lipopolysaccharide binding protein (LBP), and of general inflammation, C-reactive protein (CRP), in a unique, pre-onset study of schizophrenia. This sample was composed of 80 case-control matched pairs of US military service members from whom blood samples were obtained at time of entry to service, before a psychiatric diagnosis was made. Elevated levels of sCD14 in individuals who were subsequently diagnosed with schizophrenia generated odds ratios of 1.22 for association with disease ($p < 0.02$). Conversely, LBP levels for those who developed schizophrenia were unchanged or very marginally decreased compared to controls ($p = 0.06$). No significant changes were found for CRP in schizophrenia compared with their matched controls. This diversity of patterns suggests that a dysregulated immune system is present prior to a diagnosis of schizophrenia. In particular, sCD14 elevation and discordant LBP decrease in cases support a more generalized monocyte activation rather than a specific translocation of gut bacteria into circulation. The corresponding absence of general inflammation as measured by CRP may indicate that this monocyte activation or related immune dysfunction precedes the early inflammatory stage frequently evident in schizophrenia.

1. Introduction

A low-level systemic inflammation is a hypothesized harbinger of schizophrenia and other psychoses, as dysregulated inflammatory markers are often found in early vs. later stages of these disorders (Bechter, 2013; Li et al., 2013; Niebuhr et al., 2011; Niebuhr et al., 2008; Severance et al., 2012; Suvisaari and Mantere, 2013). It is not known if this inflammation is a source or a consequence of the disease state, but it is a condition that may be associated in part with co-occurring defects in the gastrointestinal, immune and vascular systems (Severance et al., 2015; Severance et al., 2016). The convergence of these dysfunctions is reflected in a model of the gut-brain axis by which microbial dysbiosis in the gut promotes an inflammatory environment that in turn results in compromised vascular endothelial cytoarchitecture in the intestinal tract and at the blood brain barrier. As such, this process allows for (1) the translocation of gut-derived bacteria, toxins and food peptides into systemic circulation, and (2) access of these gut-based products and associated systemic immune factors to the brain (Severance et al., 2013; Severance et al., 2015; Severance et al., 2016). As understanding and acceptance of a dynamic gut-brain interaction gains traction, an imbalanced gut microbiome may be an important environmental factor to consider in studies of the pathophysiology of schizophrenia.

Microbial dysbiosis related to schizophrenia has been documented in deep sequencing studies and biomarker examinations of microbial translocation (Castro-Nallar et al., 2015; Severance et al., 2013; Severance et al., 2015; Yolken et al., 2015). Previously, we found that people with schizophrenia had increased levels of an often-used surrogate marker for bacterial translocation, soluble CD14 (sCD14), as compared to controls (Severance et al., 2013). Recent studies, however, show that sCD14 is less specific a marker of the bacterial translocation process than lipopolysaccharide-based markers (Barbosa et al., 2012; Romero-Sanchez et al., 2012). Indeed, we found that although the

Abbreviations: AFHSB, Armed Forces Health Surveillance Branch; CRP, C-reactive protein; DMSS, Defense Medical Surveillance System; DoDSR, Department of Defense Serum Repository; ELISA, enzyme-linked immunosorbent assays; IgG, Immunoglobulin G; LBP, lipopolysaccharide binding protein; MNOPP, Military New Onset Psychosis Project; OR, odds ratio; sCD14, soluble CD14.

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lipopolysaccharide binding protein (LBP) was moderately correlated with sCD14 in both cases and controls, it was not differentially upregulated in individuals with schizophrenia, as was sCD14. Both markers were also associated with levels of C-reactive protein (CRP) only in schizophrenia, suggesting that bacterial translocation may differentially contribute in part to inflammation related to this disorder (Severance et al., 2013).

The ability to predict a forthcoming diagnosis based on a blood sample is an extremely valuable tool and gives the opportunity for early possible preventive treatment. Our longitudinal studies of a unique population cohort from the United States (US) military have enabled this possibility and led to the identification of a number of immune related biomarkers that are altered prior to a formal diagnosis of schizophrenia (Li et al., 2013; Niebuhr et al., 2011; Niebuhr et al., 2008; Weber et al., 2015). We sought to examine the hypothesis that gut permeability and monocyte activation may be part of the predictive pathogenesis of this psychiatric disorder. We tested this hypothesis by measuring sCD14 and LBP in this population and compared these marker patterns to a generalized, systemic measure of inflammation, CRP.

2. Materials and methods

2.1. Study population

We used a subset (80 cases with schizophrenia and their matched controls) of the Military New Onset Psychosis Project (MNOPP), a large nested case-control study of the US active component military service members who developed schizophrenia (855 cases and 1165 matched controls) and received medical discharges from the military between 1992 and 2005. The MNOPP study population was individually paired and matched on many demographic and technical factors, as described below. The subset includes first available serum specimens, and it was assembled so that we could test and identify promising biomarkers in a representative case-control study pilot sample. For those biomarkers deemed promising, future full cohort screening tests are planned.

The details of the MNOPP, the diagnostic process leading to medical discharge from military service and the validity of the psychiatric diagnosis have been provided elsewhere (Millikan et al., 2007; Niebuhr et al., 2008). Briefly, individuals whose stored blood samples were evaluated in this study were identified based on a retrospective analysis of physical disability and medical records. Military medical records were screened for incidences of hospitalization for a psychiatric disorder, as diagnosed using the International Classification of Disease 9th Revision (ICD-9-CM) codes (290–319).

Prospective members of the Armed Forces are medically screened at accession for both currently present, and histories of, physical and mental conditions that may be disqualifying for accession. All applicants are interviewed by a physician, and those exhibiting symptoms of mental illness are further evaluated. It is possible that applicants deny medical history or symptoms of mental illness. It is extremely unlikely that anyone floridly psychotic would be permitted to enlist. At this time, blood is drawn for human immunodeficiency virus (HIV) testing. The United States Military Entrance Processing Command (USMEPCOM), has processes in place to conduct these screenings and to identify individuals who do not meet the standards outlined in Department of Defense Instruction (DoDI) 6130.03 “Medical Standards for Appointment, Enlistment, or Induction in the Military Services.” If, at the time of this screening, a physical or mental condition is identified that may be disqualifying, the prospective member can be referred for additional assessment by a medical specialist consultant prior to a final medical qualification decision being made by USMEPCOM (Report on Preliminary Mental Health Screenings for Individuals Becoming Members of the Armed Forces, https://health.mil/Reference-Center/Reports/2017/01/11/Report-on-Preliminary-Mental-Health-Screenings).

In more detail, the MNOPP cases were selected from the data provided by each of the U.S. military disability review agencies: the Army Physical Disability Agency, the Secretary of the Navy Council of Review Boards, and the Air Force Personnel Center/U.S. Air Force Physical Disability Division. The medical and demographic data from 1989 to 2006 (released in 2007) were provided by the Defense Medical Surveillance System (DMSS), the Armed Forces Health Surveillance Branch (AFHSB), Silver Spring, MD. Serum specimens from 1988 to 2006 (released in 2007) were retrieved from the Department of Defense Serum Repository (DoDSR), AFHSB, Silver Spring, MD. The mission of the DoDSR includes storage of serum that remains following mandatory HIV- and operational deployment-related testing. Those aged 18 and older who were on active component at the time of their schizophrenia diagnosis and who had at least one serum sample of 0.5 ml or greater in the DoDSR obtained prior to diagnosis were selected as potential MNOPP cases. The time of disease onset was estimated as the earliest date of either the first hospitalization with psychiatric disorder, International Classification of Disease 9th Revision (ICD-9-CM) codes (290–319) in any diagnostic position, or the date when the medical or physical evaluation board was initiated. Control subjects, who were over the age of 18 with no inpatient or outpatient psychiatric disorder diagnoses, were selected from the active component U.S. military service population. All MNOPP control subjects were matched to their cases on sex, race, branch of military service, date of birth (±12 months), year of military entrance (±12 months), and serum specimen collection time (±90 days). Multiple matched (±90 days) serum specimens, stored at −30 °F, were obtained for the MNOPP from the DoDSR. Demographic and other study population characteristics are described in Table 1.

For this study, we have randomly (without replacement) selected a sample of 80 MNOPP subjects who later were diagnosed with schizophrenia. Their individually matched (1:1) controls were also pulled from the MNOPP study. The subjects' first available serum specimens (usually from the time of military entrance) were identified from the existing pool of sera obtained for the MNOPP and tested.

The work was performed under human subjects protocol WRAIR #2140 approved by the Walter Reed Army Institute of Research Institutional Review Board and the Institutional Review Board of the Johns Hopkins School of Medicine.

2.2. Laboratory procedures

sCD14, LBP and CRP levels were measured according to the manufacturer's protocol using commercially available kits (Human sCD14 Quantikine ELISA kit, R&D Systems, Minneapolis, MN, U.S.A.; Multispecies Lipopolysaccharide Binding Protein ELISA kit, Cell Sciences, Canton, MA, U.S.A.; High Sensitivity C-Reactive Protein ELISA kit, IBL America, Minneapolis, MN, U.S.A.). Serum dilutions were 1:200 for sCD14, 1:300 for LBP and 1:100 for CRP.

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were defined as three times and ten times the standard deviation of the blank absorbances, respectively. For the sCD14 assay, these values were 0.002 for LOD and 0.006 for LOQ. For the LBP assay, these values were 0.007 for LOD and 0.02 for LOQ. For the CRP assay, these values were 0.02 for LOD and 0.06 for LOQ. Mean level absorbances for each biomarker fell above these limits and significant differences were adequately detectable.

2.3. Data analyses

We have calculated means and p-values of the paired case-control differences of the standardized marker levels for cases and controls overall and stratified by gender. Student's *t*-test was used when the sample data were normally distributed. When sample data distributions were not normal, we used signed or signed rank test depending on the symmetry of the distribution. Spearman correlation was applied to find strength and significance of inter-variable relationships of CRP, LBP and

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