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IgG dynamics of dietary antigens point to cerebrospinal fluid barrier or flow dysfunction in first-episode schizophrenia

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ABSTRACT

Schizophrenia is a complex brain disorder that may be accompanied by idiopathic inflammation. Classic central nervous system (CNS) inflammatory disorders such as viral encephalitis or multiple sclerosis can be characterized by incongruent serum and cerebrospinal fluid (CSF) IgG due in part to localized intrathecal synthesis of antibodies. The dietary antigens, wheat gluten and bovine milk casein, can induce a humoral immune response in susceptible individuals with schizophrenia, but the correlation between the food-derived serological and intrathecal IgG response is not known. Here, we measured IgG to wheat gluten and bovine milk casein in matched serum and CSF samples from 105 individuals with first-episode schizophrenia ($n = 75$ antipsychotic-naïve), and 61 controls. We found striking correlations in the levels of IgG response to dietary proteins between serum and CSF of schizophrenia patients, but not controls (schizophrenia, $R^2 = 0.34–0.55$, $p \leqslant 0.0001$; controls $R^2 = 0.05–0.06$, $p > 0.33$). A gauge of blood–CSF barrier permeability and CSF flow rate, the CSF-to-serum albumin ratio, was significantly elevated in cases compared to controls ($p \leqslant 0.001–0.003$). Indicators of intrathecal IgG production, the CSF IgG index and the specific Antibody Index, were not significantly altered in schizophrenia compared to controls. Thus, the selective diffusion of bovine milk casein and wheat gluten antibodies between serum and CSF in schizophrenia may be the function of a low-level anatomical barrier dysfunction or altered CSF flow rate, which may be transient in nature.

1. Introduction

A variety of central nervous system (CNS) and peripheral biomarkers of inflammatory processes are altered in schizophrenia, including C-reactive protein, cytokines, kynurenine pathway metabolites, autoantibodies, antibodies to microbial agents and other extrinsic antigens, gastrointestinal (GI) and white matter functions or morphologies (Dickerson et al., 2013; Drexhage et al., 2010; Fillman et al., 2013, 2014; Gibney and Drexhage, 2013; Leonard et al., 2012; Linderholm et al., 2012; Miller et al., 2011, 2012; Monji et al., 2013; Muller, 2014; Muller et al., 2012; Severance et al., 2012a, 2013, 2014; Torrey et al., 2012; Yolken and Torrey, 2008). However, the mechanisms underlying variable immune activation observed in schizophrenia populations are poorly understood, because the immune pathology differs in scope and intensity from classic inflammatory diseases of the CNS, such as viral encephalitis and multiple sclerosis (Bechter, 2013; Bechter et al., 2010). It has been difficult to fully disentangle the contribution of antipsychotics to changes in inflammatory indices in schizophrenia, but a number of studies performed in recent onset and antipsychotic-naïve patients suggests that evidence of specific immune activation can be seen early in the disease, even before medication is administered (Beumer et al., 2012; Drexhage et al., 2010, 2011; Leonard et al., 2012; Miller et al., 2012; Mondelli and Howes, 2014; Severance et al., 2012a,b, 2013; Steiner et al., 2012; Stojanovic et al., 2014).

In schizophrenia, a subset of individuals may be particularly sensitive to immune activation following the digestion of certain dietary proteins, such as wheat gluten and bovine milk casein (Cascella et al., 2011; Dickerson et al., 2010; Dohan, 1979, 1981; Dohan and Grasberger, 1973; Dohan et al., 1969; Lachance and

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McKenzie, 2014; Niebuhr et al., 2011; Reichelt, 1991; Reichelt et al., 1981, 1995; Severance et al., 2010a). The proteins, gluten and casein, are hydrolyzed in the GI tract into hundreds to thousands of peptides, some of which have been shown to have opioid-like properties and are referred to as exorphins (Boutrou et al., 2013; Dohan, 1979, 1980, 1988a,b; Prandi et al., 2014; Reichelt, 1991, 1994; Reichelt et al., 1981, 1985, 1995, 2012). The immunomodulatory potential of these exorphins is not well-understood, with observations that among the repertoire of digested peptides, some have pro-inflammatory and others have anti-inflammatory effects (Aihara et al., 2014; Barnett et al., 2014; Haq et al., 2014; Kaminski et al., 2007). The mechanisms by which peptides derived from wheat gluten and bovine milk casein or the associated immune response might be pathogenic in schizophrenia are not known. Older studies report that casein-related exorphins are present in the CSF of individuals with post-partum depression and schizophrenia (Lindstrom et al., 1984, 1986). Presumably, exorphins located in the CSF would lead to intrathecal production of antibodies against these antigens. Intrathecal IgG production directed at specific antigens occurs in viral encephalitis, and this IgG abundance is reflected by a lack of congruence between CSF and serological IgG. In patients with multiple sclerosis, CSF immune profiles are often characterized by the chronic intrathecal production of polyspecific IgG, and similarly, serological and CSF IgG levels do not correlate (Jacobi et al., 2007; Stangel et al., 2013). These dynamics are complicated, but their evaluation can lend insight to the degree that CSF- and brain-related endothelial barrier or flow defects and the immune response to dietary antigens might be involved in the pathogenesis of schizophrenia.

In the present study, we sought to quantify the relative differences in food protein-related antibody levels and examine the possibility of an intrathecal source of antibody production in patients with first episode schizophrenia, the majority of whom were antipsychotic-naïve, compared to non-psychiatric controls. We measured IgG to bovine milk casein and wheat gluten in matched serum and CSF samples and compared antibody levels to standard indices of CSF barrier dysfunction and localized CNS antibody generation.

2. Materials and methods

2.1. Participants

Methods for identifying and characterizing individuals with a first episode of schizophrenia according to criteria defined by DSM-IV have been previously described (Leweke et al., 2004). A total of 105 individuals with first episode schizophrenia were included. Seventy-five of these individuals were antipsychotic-naïve and 30 were currently receiving antipsychotic medication. Only DSM-IV diagnoses of 295.1–295.3 were included. Sixty-one healthy volunteers served as the control group. Individuals were excluded from the study if they had a relevant comorbidity such as heart disease, liver cirrhosis, known immune-mediated disease (such as multiple sclerosis), or a history of substance dependence. Only individuals who were less than 50 years of age were included. Demographic data regarding age and sex are listed in Table 1. Previous analyses of this study population indicated that study individuals were also generally homogeneous in terms of socioeconomic characteristics, geography and ethnicity and did not differ significantly with respect to body mass index (Hayes et al., 2014; Leweke et al., 2004). Informed written consent was obtained from all study participants. Protocols for sample collection and analyses were approved by the ethics committee at the University of Cologne, Heidelberg University and Johns Hopkins University, in accordance with the Declaration of Helsinki.

Table 1
Study population demographics.

	n	Age Mean years ± SEM[a]	Female *n* (%)
Controls	61	27.16 ± 0.65	31 (50.8)
First episode schizophrenia	105	28.62 ± 0.78	38 (36.2)
Antipsychotic-naive	75	28.53 ± 0.92	26 (34.7)
Antipsychotic-positive	30	28.87 ± 1.53	12 (40.0)

[a] SEM refers to standard error of the mean.

Serum and CSF samples were collected according to the methods described previously (Leweke et al., 2004). Lumbar punctures were performed at the same time of day using a non-traumatic lumbar puncture procedure. Serum samples were collected concurrently. Serum and CSF analyses performed at time of acquisition included measures of albumin, total IgG and glucose. Samples were then frozen and stored at −80 °C.

2.2. Laboratory procedures

The enzyme-linked immunosorbent assays (ELISAs) to detect bovine casein-, and wheat gluten-related IgG have been previously described (Severance et al., 2012a,b). Whole casein was purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.). Whole gluten was extracted from the wheat cultivar Cheyenne as previously described (Samaroo et al., 2010). In brief, for both the casein and gluten immunoassays, plate wells were incubated with 100 ng protein in 50 µl carbonate buffer (0.05 M carbonate–bicarbonate, pH 9.6; Sigma–Aldrich, St. Louis, MO, U.S.A.) overnight at 4 °C, and plates were blocked for 1 h at 37 °C with 1% (wt/vol) human serum albumin (Sigma–Aldrich, St. Louis, MO, U.S.A.) in PBS. Plates were then incubated with serum samples diluted 1:200 and CSF samples diluted 1:10 for 2 h at 37 °C. Plates were washed and incubated with peroxidase-conjugated goat-anti-human IgG secondary antibodies for 30 min at 37 °C (Southern Biotech, Birmingham, AL, U.S.A.). A 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate) and 0.02% hydrogen peroxide solution (KPL Protein Research Products, Gaithersburg, MD, U.S.A.) was added for color development, and absorbance was measured at 405 nm, with a reference wavelength of 490 nm, in an automated microtiter plate reader (Molecular Devices, Menlo Park, CA, U.S.A.).

2.3. Statistical analyses

Plate-to-plate variation of the food antigen IgG was corrected by mean-normalizing each plate (i.e. mean absorbances of each plate equaled a value of "1"). Both mean-normalized and non-mean-normalized data for food antigen IgG were subjected to statistical analyses. Background reactivity of blank wells was subtracted from plate measurements for the non-mean-normalized dataset. When results were consistent across both datasets, the mean-normalized data were used to depict representative results in the relevant tables and figures. Quantitative biomarker levels (antibodies, glucose, albumin) were compared between study groups using two tailed *t*-tests. Multiple linear regression models including age and sex were implemented to assess biomarker inter-correlations and associations of biomarkers with other variables. Bonferroni correction of multiple comparisons resulted in *p* values less than 0.0125 (0.05/4) to be considered statistically significant. Information regarding albumin, total IgG and glucose in body fluids was available for only a subset of individuals in each group, and these sample sizes are listed in the results section.

The albumin and total IgG measures and interpretations described here are considered standard indices of CSF activity (Kirch et al., 1992; Mundt and Shanahan, 2011; Reiber, 1994;

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