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Anti-brain autoantibodies in the serum of schizophrenic patients: A case-control study

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ABSTRACT

Schizophrenia is considered a neurodevelopmental disorder with a multifactorial pathogenesis where autoimmune factors may play a significant role. The aim of this study was to verify the presence of anti-brain autoantibodies in the serum of schizophrenic patients compared to healthy controls. Autoantibodies against brain were detected by the immunofluorescence method, utilizing sections of rat hippocampus and hypothalamus and of monkey cerebellum. Three different fluorescence patterns were observed, staining the nucleus-cytoplasm of neurons, the neuroendothelial of blood vessel and the neurofilaments. Search for other organ-specific and non organ-specific autoantibodies was performed in all sera by indirect immunofluorescence method, enzyme linked immunosorbent assay and chemiluminescence immunoassay. Results showed a significant association between schizophrenia and anti-brain autoantibodies against the neuroendothelium of blood vessel in hypothalamus, hippocampus and cerebellum; a significant nuclear and cytoplasmic staining of neurons was assessed only for the hippocampus. No other significant association was found, except between schizophrenia and anti-nuclear autoantibodies on HEp-2 cells. In conclusion, these results support the hypothesis of a significant association between schizophrenia and circulating anti-brain autoantibodies, suggesting a diffuse reactivity against the neuroendothelium of blood vessel and highlighting a nuclear and cytoplasmic staining of the neurons of hippocampus.

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1. Introduction

Schizophrenia is a chronic and debilitating disease that affects approximately 1% of the world population. A considerable amount of research has been focused on the etiopathogenesis of schizophrenia, but none of the current theories have been considered fully exhaustive for the explanation of its complexity and clinical heterogeneity (Tandon et al., 2008; Rogers and Goldsmith, 2009; Schmidt-Kastner et al., 2012).

Many authors have actively investigated a role for autoimmune dysfunction since the 1930s, when autoantibodies were first reported in a schizophrenic patient (Amital and Shoenfeld, 1993; Goldsmith and Rogers, 2008; Yum et al., 2009; Kayser and Dalmau, 2011). To explain how the immune dysregulation may be an etiological factor in schizophrenia, some authors have referred to the pre and perinatal exposure to adverse factors. These may be responsible for fetal neuroinflammation, which might cause a synergistic impairment of the normal developmental trajectories of both the immune system and the central nervous system (CNS), inducing a predisposition to long-term brain pathology (Winter et al., 2009; Kinney et al., 2010).

Immunological alterations in schizophrenia may be conceptualized along one or a combination of the following three tracks: a diffuse, non-specific over-activation of the immunological response system, a T-helper cell type 1 immune activation and a T-helper cell type-2 immune activation (Strous and Shoenfeld, 2006). The type-1 immune system promotes the cell-mediated immune response directed against intracellular pathogens, whereas the type-2 response helps B-cell maturation and promotes the humoral immune response, including the production of

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antibodies directed against extracellular pathogens (Muller and Schwarz, 2010).

In their review Jones et al. examined several studies reporting an increased level of autoantibodies against specific areas of the brain or against brain constituents in the sera of patients with schizophrenia when compared to controls, but consistency in the findings between different research groups was not high. Probably, the intrinsic variety of phenotypes of schizophrenia, the different diagnostic criteria used in the assessment of patients, the different types of autoantibodies searched out as well as the different substrates and laboratory methods used, may have influenced this heterogeneity. However, they assumed that compelling evidence exists in support of the hypothesis that autoantibodies may play a role in the development of at least some cases schizophrenia (Jones et al., 2005).

On the other hand, in recent years several authors have provided a lot of evidence on a severe form of encephalitis, characterized by psychotic symptoms, motor changes such as catatonia, seizure-like activity and dyskinesias, associated with autoantibodies against NR1 and NR2 subunits of the *N*-methyl-D-aspartate (NMDA) receptor (Barry et al., 2011; Peery et al., 2012; van de Riet and Schieveld (2013)); other authors have proposed an indirect pathogenetic role, via the NMDA receptor, of the α-actinin-2 as an autoantigen target in a CNS diseases, such as schizophrenia, epilepsy, ischemic brain damage, neurodegenerative disorders and CNS lupus (Oikonomou et al., 2011).

In the context of the ongoing debate regarding the role of autoimmune factors in the pathogenesis of schizophrenia, we present a case-control study designed to test the hypothesis of a significant association between specific anti-brain autoantibodies and schizophrenia. The objective was to compare the presence of circulating autoantibodies between a group of patients suffering from schizophrenia and a group of healthy controls, both with negative clinical history for autoimmune diseases. We therefore investigated both the organ-specific and non organ-specific autoimmunity. Within the organ-specific autoimmunity, we focused on anti-brain autoantibodies, directed against three different areas that are functionally involved in the pathogenesis of the disease: hippocampus, hypothalamus and cerebellum (Harrison, 2004; Andreasen and Pierson, 2008; Tamminga et al., 2010). For each of these three brain areas, different fluorescence patterns were examined, which corresponded to nuclear and cytoplasmic staining of the neurons, neuroendothelium of blood vessels and neurofilaments.

2. Methods

2.1. Subjects

The study group was composed of 30 patients with schizophrenia, recruited from consecutive admission at the Psychiatric Unit, Department of Neuroscience and Sensory System, University "Aldo Moro" of Bari, Italy, over a six months period (from June to December 2008). There were 17 males and 13 females with an average age of 44 years (range 26–65). Diagnosis of schizophrenia met the diagnostic criteria in the DSM-IV TR and was made by expert psychiatrists in clinical-diagnostic assessments. The Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) measured the presence and severity of psychiatric symptoms; Clinical Global Impression (CGI) (Guy, 2000) was used to rate the current severity of the patient's illness. Patients with schizophrenia were also subjected to clinical and instrumental assessment including anamnesis, examination of previous medical records, blood-chemical investigations and electrocardiogram.

The control group was composed of 39 healthy, normal volunteers, recruited from the staff of the Laboratory of Clinical Pathology, University Hospital of Bari.

There were 14 males and 25 females with an average age of 42 years (range 28–55). The selection of this control group was made through clinical assessment of psychopathological conditions and an anamnestic interview, making them eligible only upon the exclusion of a current and lifetime major psychiatric disorder.

All subjects, patients and controls, were screened for the absence of a previously diagnosed autoimmune disease after a careful evaluation of the medical history and physical examination.

A written, informed consent was obtained after the study procedures had been fully explained by the research team.

Ethical committee of the Hospital Consortium Policlinico of Bari, Italy approved the study (prot. n. 1040/C.E.01/10/2009).

2.2. Methods

All sera were separated from peripheral blood samples and frozen at −70 °C until assayed. All samples were analyzed in a single analytical session.

Organ-specific (including anti-brain) and non organ-specific autoantibodies were assayed by indirect immunofluorescence (IIF), enzyme linked immunosorbent assay (ELISA) and chemiluminescence immunoassay (CLIA), as explained below.

2.2.1. Organ-specific anti-brain autoantibodies

The detection of anti-brain autoantibodies was performed with the IIF method using kits provided free of charge by BioSystem (Barcellona, Spain).

In detail, each sample was analyzed at a serum dilution of 1:10 on two different substrates of rat's brain, dentate gyrus of hippocampus and hypothalamus, and on two different sections of monkey cerebellum, vermis and floccule.

The slides were provided as frozen sections fixed with acetone, and without fixative.

Anti-human Ig conjugate was used.

Different fluorescence patterns can be observed in these sections

a) positive reaction with nuclear and cytoplasmic staining of neurons;
b) a positive reaction of the neuroendothelium of blood vessels; and
c) a positive reaction of the neurofilaments.

A positive and a negative control serum were analyzed in each analytical session. The intensity of the fluorescence was determined by two different readers blind to subject characteristic and a scale from 0 to 3 was assigned (0 no reaction, 1 weak or uncertain fluorescence, 2 moderate fluorescence, 3 high fluorescence). Samples scored positive if a 2 or 3 fluorescence reaction was observed at 1:10 sample dilution.

2.2.2. Other organ-specific autoantibodies

Anti-mitochondrial autoantibodies (AMA), anti-smooth muscle autoantibodies (ASMA), anti-parietal cell autoantibodies (APCA) were assayed by the IIF method at a serum dilution of 1:40, using frozen sections of rat (liver, kidney and stomach) and an anti-human IgG conjugate (Euroimmun, Lubueck, Germany).

Samples scored positive if a fluorescence reaction was observed at 1:40 sample dilution.

Anti-thyroperoxidase (TPO) and anti-thyroglobulin (Tg) autoantibodies were assayed with the CLIA method with the LIASION analyzer (DiaSorin, Saluggia, Italy).

Samples scored positive if autoantibody levels exceeded the cut-off value provided by the manufacturer (60 U/mL for anti-TPO and for anti-Tg).

Anti-tissue transglutaminase IgA (tTG-IgA) and IgG (tTG-IgG) autoantibodies were assayed using the ELISA method on the Impatto analyzer (Eurospital, Trieste, Italy).

Samples scored positive if autoantibody levels exceeded the cut-off value provided by the manufacturer (9 U/mL for anti-tTG IgA e 30 U/mL for anti-tTG IgG).

2.2.3. Non organ-specific autoantibodies

Anti-nuclear autoantibodies (ANA) were assayed with the IIF method at a serum dilution of 1:80, using human epithelial cells (HEp-2) as substrate and an anti-human IgG conjugate (Euroimmun).

Samples scored positive if a fluorescence reaction was observed at 1:160 dilution.

All ANA-positive samples were investigated for anti-dsDNA and anti-extractable nuclear antigen (ENA) autoantibodies, by the LIASION and ETIMAX analyzers (DiaSorin), respectively. Samples scored positive if autoantibodies levels exceeded the cut-off value provided by the manufacturer (25 U/mL for anti-dsDNA and index > 1.1 for anti-ENA).

Anti-cardiolipin autoantibodies (aCL-IgG/IgM) were assayed with the EIA method with ETIMAX 3000 (DiaSorin). Samples scored positive if autoantibodies levels exceeded the cut-off value provided by the manufacturer (20 U/mL for aCL-IgG and 15 U/mL for aCL-IgM).

Anti-neutrophil cytoplasmic autoantibodies (ANCA) were assayed by the IIF method at serum dilution of 1:20, using granulocytes fixed with ethanol and formaldehyde and an anti-human IgG conjugate (Euroimmun). Samples scored positive if a fluorescence reaction was observed at 1:20 sample dilution.

Anti-cyclic citrullinated peptide autoantibodies (anti-CCP2) were assayed with the ELISA method with ETIMAX 3000 (DiaSorin). Samples scored positive if autoantibody levels exceeded the cut-off value provided by the manufacturer (25 U/mL).

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