European Neuropsychopharmacology (2015) 25, 2326–2332

www.elsevier.com/locate/euroneuro

SHORT COMMUNICATION

No evidence for the presence of neuronal surface autoantibodies in plasma of patients with schizophrenia

Hans C. van Mierlo[a,*,1], Marleen H. van Coevorden-Hameete[b,1], Leon P. Munting[b], Esther de Graaff[b], Lot de Witte[a], the GROUP investigators[2]

[a]*Department of Psychiatry, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands*
[b]*Department of Biology, Division of Cell Biology, Utrecht University, The Netherlands*

Received 9 June 2015; received in revised form 5 August 2015; accepted 24 September 2015

KEYWORDS
Schizophrenia;
Autoimmune;
Antibodies;
Encephalitis;
Psychosis;
Immunology

Abstract
The immune system has been implicated in the etiology of schizophrenia. Autoimmunity by antibodies against neuronal cell surface antigens has been proposed as one of the pathological mechanisms. We examined plasma samples of 104 patients diagnosed with schizophrenia for the presence of autoantibodies against neuronal cell surface antigens using cultured hippocampal neurons and transfected HeLa cells. None of the samples tested positive for the presence of these autoantibodies. Based on our results it seems unlikely that autoantibodies against neuronal cell surface antigens play a role in the pathogenesis of schizophrenia, although further studies using cerebrospinal fluid are needed.

*Corresponding author. Tel.: +31 887558180; fax: +31 887555466.
E-mail address: h.vanmierlo@umcutrecht.nl (H.C. van Mierlo).
[1]Both authors contributed equally to this work.
[2]Genetic Risk and Outcome of Psychosis (GROUP) Investigators
Richard Bruggeman[1], Wiepke Cahn[2], Lieuwe de Haan[3], René S. Kahn[2], Carin J. Meijer[3], Inez Myin-Germeys[4], Jim van Os[4], Durk Wiersma[1]
[1]University Medical Center Groningen, Department of Psychiatry, University of Groningen, The Netherlands
[2]University Medical Center Utrecht, Department of Psychiatry, Brain Center Rudolf Magnus, The Netherlands
[3]Academic Medical Center University of Amsterdam, Department of Psychiatry, Amsterdam, The Netherlands
[4]Maastricht University Medical Center, South Limburg Mental Health Research and Teaching Network, EURON, Maastricht, The Netherlands

http://dx.doi.org/10.1016/j.euroneuro.2015.09.017
0924-977X/

1. Introduction

Different lines of evidence from the genetic (Ripke et al., 2014), epidemiological (Benros et al., 2014) and the immunological field (Tomasik et al., 2014) suggest that the immune system is involved in the pathogenesis of schizophrenia. However, the exact pathological mechanism explaining how the immune system contributes to schizophrenia is still unknown. In the 1960s autoimmunity was already proposed as a potential pathological mechanism (Heath and Krupp, 1967). More recent support for this theory is provided by studies showing a higher prevalence of autoimmune disorders in patients with schizophrenia (Benros et al., 2014) and the association of schizophrenia with genetic loci that are involved in adaptive immune responses and autoimmunity, including the MHC region (Ripke et al., 2014; The Network and Pathway Analysis Subgroup of the Psychiatric Genomics Consortium, 2015).

One of the key mechanisms involved in autoimmune disorders is the production of self-reactive antibodies, the so-called autoantibodies. Some neurological autoimmune disorders, such as anti-NMDA receptor encephalitis, are caused by autoantibodies targeting neuronal cell surface antigens. These pathogenic autoantibodies can impair the function of their target protein in various ways, for example by clustering and internalization of the receptor leading to diminished cell surface expression (for review see van Coevorden-Hameete et al. (2014)). This leads to changes in synaptic transmission and neuronal excitability, resulting in neurologic and/or psychiatric symptoms. Isolated episodes with psychotic symptoms can occur in patients with anti-NMDA receptor encephalitis (Kayser et al., 2013). It has therefore been postulated that neuronal surface autoantibodies could cause a clinical syndrome similar to schizophrenia (Coutinho et al., 2014).

Several groups have investigated the presence of neuronal autoantibodies in patients with schizophrenia. The presence of antibodies against brain antigens in general has been examined by incubating rat brain tissue slices with patients' sera. Anti-brain antibodies were found to be increased in some studies but not in others, thoroughly reviewed elsewhere (Jones et al., 2005). The last decade, multiple studies have focused on the seroprevalence of autoantibodies targeting specific neuronal surface antibodies, such as NMDA (Pearlman and Najjar, 2014), dopamine, AMPA and GABA receptor antibodies (Ezeoke et al., 2013; Müller et al., 2014), with inconclusive evidence.

We hypothesized that known and/or yet unknown autoantibodies against neuronal cell surface antigens are involved in the pathogenesis of schizophrenia in a subgroup of patients. In this study we therefore set out to examine the prevalence of neuronal autoantibodies by screening plasma of 104 patients with schizophrenia using live rat hippocampal neurons. In addition, cell-based assays (CBA) were used to test these samples for autoantibodies against a selection of 24 neuronal cell surface antigens that have been associated with schizophrenia in GWAS studies (Ayalew et al., 2012; Greenwood et al., 2011; Levinson et al., 2011; Sullivan et al., 2008).

2. Experimental procedures

2.1. Participants

Plasma samples from a subgroup of 104 patients diagnosed with schizophrenia and schizophreniform disorder that participated in the Genetic Risk and Outcome of Psychosis (GROUP) study in the Netherlands were used for this study. Further details of this study are described elsewhere (Korver et al., 2012). In brief, inclusion criteria for patients participating in the original study were: (1) Age range of 16 to 50 years, (2) a diagnosis of non-affective psychotic disorder according to DSM-IV criteria, (3) good command of the Dutch language, (4) and able and willing to give written informed consent. A plasma sample of a patient with autoimmune encephalitis caused by anti-GluR1 antibodies was included as positive control.

2.2. Commercial antibodies

The following antibodies were used in this study: mouse anti-myc (Santa Cruz Biotechnology, 9E10), mouse anti-v5 (Life Technologies, R960-25) and Alexa 488- and Alexa 568- conjugated anti-mouse and -human IgG secondary antibodies (Life Technologies).

2.3. DNA constructs

DNA constructs for the 24 candidate antigens, including a tag to identify transfected cells, were either present in our lab, or a gift from other laboratories. Table 1 depicts the specifications of all DNA constructs used in this study.

2.4. Immunocytochemistry of live primary hippocampal neurons

Cultures of primary hippocampal neurons were prepared from embryonic day 18 rat brains. Neurons were grown in Neurobasal medium (Life Technologies) supplemented with B27, 0.5 μM glutamine, 12.5 μM glutamate and penicillin/streptomycin. Neurons were plated on coverslips coated with poly-L-lysine (30μg/ml) and laminin (2 μg/ml) at a density of 75,000/well. Neurons were then incubated with plasma (1:50) in unconditioned medium for 1 hour at 37 °C, washed in medium and fixed for 10 min with 4% paraformaldehyde (PFA)/4% sucrose at room temperature. Cells were incubated with the secondary-antibody in GDB buffer (0.1% bovine serum albumin (BSA), 0.4 M NaCl, 15 mM phosphate buffer, pH 7.4) for 1 h at room temperature. Neurons were then washed in PBS and mounted on slides in Vectashield mounting medium containing DAPI (Vector Laboratories).

2.5. Cell-based assay

HeLa cells were cultured in Ham's F10/DMEM (50%/50%) containing 10% fetal calf serum and 1% penicillin/streptomycin. HeLa cells were detached using trypsin/EDTA and were plated on 16 well Tissuetek chamber slides (Thermoscientific) and were transfected using Polyethylenimine (1 mg/ml, PEI max, Polysciences, 24765-2). Cells were fixed for 10 min using 4% PFA and incubated overnight at 4 °C with patient plasma (1:400) in PBS+ (PBS with 1% BSA and 0,1% triton). Cells transfected with Grik3 or KCNIP constructs were also incubated with mouse anti-myc tag or mouse anti-v5 tag primary antibodies. After washing, cells were incubated with secondary antibodies in PBS+ for 1 hour at room temperature. Cells were washed and mounted in Vectashield mounting medium containing DAPI (Vector Laboratories).