Neuroscience Research 72 (2012) 87–93

Contents lists available at SciVerse ScienceDirect

Neuroscience Research

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

Monocytic HLA DR antigens in schizophrenic patients

Daniela Krause[a,*,1], Jenny Wagner[a,1], Judith Matz[a,1], Elif Weidinger[a,1], Michael Obermeier[a,1], Michael Riedel[a,1], Rudolf Gruber[b,2], Markus Schwarz[a,1], Norbert Mueller[a,1]

[a] Department of Psychiatry, Ludwig-Maximilians University Munich, Nußbaumstr. 7, 80336 Munich, Germany
[b] Department of Internal Medicine, Ludwig-Maximilians University Munich, Nußbaumstr. 7, 80336 Munich, Germany

ARTICLE INFO

Article history:
Received 24 April 2011
Received in revised form
13 September 2011
Accepted 15 September 2011
Available online 22 September 2011

Keywords:
HLA DR
Immune system
Monocytes
Infections
Poly I:C
Schizophrenia

ABSTRACT

A genetic association of specific human leukocyte antigens (HLA) DR genes and schizophrenia has recently been shown. These HLA play a fundamental role in the control of immune responses. Furthermore infectious agents have been proposed to be involved in the pathogenesis of schizophrenia. In this study we investigated the rate of HLA DR positive monocytes in schizophrenic patients compared to controls with a special focus on the adaption to in vitro stimulation with toll-like receptor ligands.

Patients with schizophrenia and matched controls were included. For each individual, we evaluated the rate of HLA DR positive monocytes (either incubated at 37 °C or after stimulation with lipopolysaccharide or Poly I:C).

We found a significantly higher percentage of schizophrenic patients with elevated HLA DR positive cells ($p = 0.045$) as compared to controls. The adjustment rate from baseline levels of monocytic HLA DR positive cells to stimulation with Poly I:C was significantly lower in schizophrenic patients ($p = 0.038$).

The increased monocytic HLA DR in schizophrenic patients and the maladjustment of their monocytic HLA DR levels to an infectious stimulus might be a sign for a disturbed monocytic immune balance in schizophrenic individuals.

1. Introduction

Human leukocyte antigens (HLA) are located on the cell membrane of all nucleated cells and play a fundamental role in the control of immune response by lymphocytes (Sette et al., 1987). The HLA molecules fulfill their biological role by modulating immune responses to foreign antigens. There are two different classes of HLA: HLA class one antigens (A, B and C) present peptides from inside the cell and control cell-mediated immune responses. HLA class two antigens (DR, DP, and DQ) present antigens derived from extracellular proteins, which have undergone endocytosis. These particular HLA/antigen complexes stimulate T helper cells to release cytokines that either activate antigen-presenting cells or if it is a macrophage destroy the antigen, or promote antibody production if the antigen-presenting cell is a B lymphocyte.

Just recently HLA became particularly important in schizophrenia research, because studies investigating genome wide scans found significant associations with schizophrenia and markers spanning the major histo-compatibility complex (MHC) region on chromosome 6p21.3-22-1, implicating the MHC region is consistent with an immune component to the risk of developing schizophrenia (Stefansson et al., 2009). In addition, also the international schizophrenia consortium has demonstrated that there is an association between the major histo-compatibility complex and schizophrenia, showing etiological mechanisms involving autoimmunity and infections (Purcell et al., 2009). The most frequently reported association between HLA and schizophrenia was HLA DRB1*0 or its alleles (Sasaki et al., 1999; Arinami et al., 1998). Akaho et al. (2000) have provided a pooled data analysis of the Japanese genotyping data and reported a significantly increased frequency of HLA DRB1 alleles in 588 patients with schizophrenia when compared to 942 controls. Within the Japanese population, several studies have consistently shown a higher frequency of HLA DR1 in patients with schizophrenia than in healthy controls; the frequencies of subjects with HLA DR1 expression was 22% versus 10% (Miyanaga et al., 1984), 23% versus 10% (Sasaki et al., 1994), 15% versus 9% (Arinami et al., 1998), and 16% versus 11% (Sasaki et al., 1999) in patients and comparison subjects. In addition, an

Abbreviations: BFA, Brefeldin A; CD, cluster of differentiation; CK, creatine kinase; CPDA, citrate phosphate dextrose adenine; CRP, C-reactive protein; DSM-IV, diagnostic and statistical manual; HLA, human leukocyte antigens; IL, interleukin; LPS, lipopolysaccharide; MHC, major histo-compatibility complex; PANSS, positive and negative symptoms scale; PFA, paraformaldehyde; Poly I:C, polyinosinic:polycytidylic acid; TLR, toll-like receptor.

* Corresponding author. Tel.: +49 89 5160 5511; fax: +49 89 5160 5774.
E-mail address: Daniela.Krause@med.uni-muenchen.de (D. Krause).
[1] Tel.: +49 89 5150 5512.
[2] Tel.: +49 89 5160 2400.

0168-0102/$ – see front matter © 2011 Elsevier Ireland Ltd and the Japan Neuroscience Society. All rights reserved.
doi:10.1016/j.neures.2011.09.004

increased frequency of HLA DR1 expression in 107 Scottish patients with schizophrenia compared with 264 blood donors has been demonstrated (Blackwood et al., 1996).

Although the mechanism of the association of HLA DR and schizophrenia is not exactly known, Narita et al. (2000) suggest prenatal infections might be involved, as they observed a significantly higher incidence of births in February and March in patients with HLA DR1. These findings are in line with epidemiological studies that revealed different environmental factors such as winter and spring birth, birth in an urban area, and complications during delivery as risk factors for schizophrenia (Wright et al., 1995; Machon et al., 1983). Moreover it has been suggested that environmental factors, such as prenatal infections, are involved in the etiology of some cases of schizophrenia (Brown, 2006). Recent studies discuss the role of postnatal infections on the pathogenesis of this disease (Yolken and Torrey, 2008).

All the above-mentioned results derive from genome analyses and therefore the precise HLA protein occurrence cannot be answered with these findings. In order to find out more about the underlying mechanism of the association between HLA DR and schizophrenia in the Caucasian population we investigated whether specifically the occurrence of HLA DR antigens differs in schizophrenic patients compared to healthy controls. No genome analyses were performed in our study population. Instead we have set a special focus on the monocytic system because these immunological cells have been identified with abnormalities in schizophrenia (Chang et al., 2010; Na and Kim, 2007). In addition, a high inflammatory set point of circulating monocytes at the transcriptome level was observed, involving various inflammatory transcripts forming distinct fingerprints (Drexhage et al., 2010). As infectious agents seem to be involved in the pathogenesis of schizophrenia, the impact of in vitro stimulation with toll-like receptor (TLR) ligands like lipopolysaccharide (LPS), representing a bacterial infection and polyinosinic:polycytidylic acid (Poly I:C), representing a viral infection, on HLA DR antigens in monocytes was evaluated.

2. Materials and methods

2.1. Characterization of the patient and control population

For this study 31 patients with schizophrenia (aged 36.5 ± 13.4 years; 18 males and 13 females) were diagnosed by two experienced psychiatrists using a structured assessment according to the diagnostic criteria, as defined by the IV edition of the diagnostic and statistical manual (DSM-IV). Patients were recruited through the Department of Psychiatry of the Ludwig-Maximilians University Munich and were hospitalized. At the time of study inclusion patients were rated with the positive and negative symptoms scale (PANSS) and showed a mean value of 92.1 (SD = 20.3). Healthy control subjects were recruited via advertisement. 30 people of the control population (aged 33.7 ± 16.1 years; 18 males and 12 females) were matched to the schizophrenic group considering gender, ethnicity and age. Controls and patients were all Caucasians living in Bavaria, Germany. All study participants gave their informed consent prior to study inclusion. The responsible authorities approved the procedure for sample collection and analysis, in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. A concomitant organic disease or acute general or genitor-urinary infections were exclusion criteria. In addition, the control group did not meet the criteria for DSM-IV diagnosis of mental illness; an experienced psychiatrist evaluated this issue. Furthermore blood analyses were performed (differential blood count, urea and electrolytes, CK, renal-, pancreatic-, liver- and thyroid-parameters, CRP and haptoglobin as an indicator for infections) in order to exclude controls with organic diseases. In the schizophrenic group, antipsychotic treatment had to be stopped at least 4 weeks prior to study inclusion. In regard to smoking habits, 58.1% of schizophrenic patients and 20.0% of controls were smokers at the time of study inclusion. Table 1 shows the characterization of the study population.

2.2. Blood samples

Blood samples were taken from schizophrenic individuals before antipsychotic medication was administered. The samples were obtained from the participants by venipuncture with one 8.5 ml CPDA-Monovette (Sarstedt, Nümbrecht, Germany) in the morning.

2.3. Antibodies

For detection of HLA DR the following fluorescending monoclonal antibodies were obtained from the manufactures: anti-HLA-DR antibody (Cy5.5), isotype IgG2b (mouse) (PE-Cy 5.5) (all Caltac Laboratories, San Francisco, CA, USA), isotypecontrol (PE), isotype IgG1κ (rat) (eBioscience, San Diego, CA, USA); anti-CD-33 antibody Cy 5.5, isotype IgG1κ (mouse); anti-CD-45 antibody allophycocyanine APC, isotype IgG1 (mouse); and anti-CD-14 antibody (FITC), isotype IgG2a (mouse).

2.4. In vitro stimulation

In order to find out more about the properties of HLA DR antigens in monocytes in regard to infectious agents, we used two TLR ligands: Poly I:C which can be considered a synthetic analog of viral double-stranded RNA. In another approach LPS was used. LPS is a major component of the outer membrane of gram-negative bacteria and it acts as an endotoxin that can elicit strong immune responses. As LPS is of crucial importance to gram-negative bacterial cells, it was therefore used to simulate bacterial infections.

4 × 1 ml CPDA-blood was treated as follows:

(a) Storage at room temperature (ca. 22 °C) for 4 h.
(b) Storage in the incubator (Memmert type U25, Heinrich Rhode GmbH, Kirchheim, Germany) at 37 °C for 4 h, addition of 10 μl/ml Brefeldin A (BFA) after 2 h of incubation.
(c) Stimulation with 1 μl LPS per 1 ml of blood (lipopolysaccaride, stored at 4 °C (Sigma, St. Louis, MO, USA)), incubation in the incubator at 37 °C for 4 h, addition of 10 μl/ml BFA after 2 h of incubation.
(d) Stimulation with 50 μg/ml Poly I:C (polyinosinic:polycytidylic acid or polyinosinic–polycytidylic acid sodium salt polyI:C, sterile dilution 10 mg and 10 ml NaCl (150 mM), 100 μl aliquoted in 1.5 ml Microtubes (Sigma, St. Louis, MO, USA)), incubation in the incubator at 37 °C for 4 h, addition of 10 μl/ml BFA after 2 h of incubation.

2.5. Staining

Staining was performed using a protocol adapted from previously described methods (Maino et al., 2007; Gruber et al., 1993). Ninety micro liter of each 1 ml incubated and/or stimulated CPDA-blood was used for staining in different tubules and 20 μl antibodies were added. Cell concentration was 10^6–10^7/ml. We used four-colour immunofluorescence staining with different antibody combinations (for details see Supplementary section). Antibody concentrations were titrated under standardized conditions (cell concentration, volume, time, wash steps) to find only such concentrations for highly affine antigen–antibody bounding. Extracellular anti-CD-antibodies helped to identify and specify