Immunobiology 218 (2013) 738–744

Contents lists available at SciVerse ScienceDirect

Immunobiology

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

Paradoxical downregulation of HLA-A expression by IFNγ associated with schizophrenia and noncoding genes

Songhai Gu[a,1], Barbara Fellerhoff[a,b,1], Norbert Müller[c], Barbara Laumbacher[a], Rudolf Wank[a,*]

[a] Immunotherapy Research Center IMMUNIS e.V., Muenchen, Germany
[b] Institut fuer Immunologie, Universitaet Muenchen, Germany
[c] Psychiatric Hospital, Universitaet Muenchen, Germany

ARTICLE INFO

Article history:
Received 16 May 2012
Received in revised form 17 August 2012
Accepted 19 August 2012

Keywords:
Downregulation
HLA-A
HLA-J pseudogene
IFNγ
Schizophrenia

ABSTRACT

Neuronal MHC/HLA regulates the synapses of the central nervous system (CNS). The expression of MHC/HLA is, in turn, regulated by immune cytokines. We were therefore interested in the regulation of schizophrenia-associated HLA antigens, specifically their regulation of expression by interferons. We had previously observed a moderately increased frequency of HLA-A10 expression in schizophrenic patients. While searching for the "true" disease gene near the HLA-A gene, we discovered that homozygosity of the *HLA-J M80469* pseudogene allele, in combination with HLA-A10 or HLA-A9, was associated with a high risk of schizophrenia (HLA-A10 relative risk = 29.33, $p = 0.00019$, patients $N = 77$, controls $N = 214$). The allele *HLA-J M80468*, which codes for interferon-inducible mRNA, conferred protection on carriers of HLA-A9 and HLA-A10 (HLA-A10 relative risk = 0.022, $p = 0.00017$). Functional analysis revealed that interferon γ (IFNγ) downregulated the expression of HLA-A9 and HLA-A10 in monocytes from *HLA-J M80469* homozygous patients but not from carriers of the *HLA-J M80468* allele. This is the first demonstration of an inverse effect of IFNγ on HLA expression that is associated with non-coding gene variants and schizophrenia. Our findings suggest that the interferons secreted during acute and chronic infections may interfere in synaptic regulation via neuronal HLA and that this disturbance in synaptic regulation may induce the symptoms of mental illness.

Introduction

Schizophrenia is a devastating mental disease. Among discussed pathogenic factors, genetic disposition has been considered to be a major factor (Kety et al. 1976). Numerous linkage studies have suggested the presence of schizophrenia susceptibility genes on chromosome 6p (Wang et al. 1995; Schwab et al. 1995). Schizophrenia-associated genetic factors located on other chromosomes and not in the chromosomal region 6p appear to be of secondary importance (Shi et al. 2009). The HLA region is comprised of highly polymorphic immune response genes and other genes with known or unknown functions. It has been shown that HLA class I genes function as both initiators of immune responses and modulators of synaptic activity (Boulanger and Shatz 2004). Previously, we reported a significant but moderate increase in the frequency of HLA-A10 expression in patients with schizophrenia (compared to healthy controls) in two independently sampled patient groups (Laumbacher et al. 2003). This prompted us to examine genes flanking the HLA region. We analyzed the frequency of *HLA-J* alleles in patients and healthy controls because HLA-J is located only 50 kb away from HLA-A (Fan et al. 1996; Ragoussis et al. 1989; Messer et al. 1992; http://hla.alleles.org/alleles/class1.html; http://hla.alleles.org/data/txt/j_gen.txt). Furthermore, only one of the alleles, *HLA-J M80468*, codes for interferon-inducible mRNA transcripts (Messer et al. 1992). Therefore, we analyzed possible functional interactions between *HLA-J* variants and schizophrenia-associated HLA-A antigens by stimulating mononuclear cells of the peripheral blood (PBMC) with IFNγ. We determined the MHC expression of monocytes from patients and control individuals and looked for correlations between schizophrenia, HLA-A expression and *HLA-J* variants.

Abbreviations: BDNF, brain-derived neurotrophic factor; HLA, human leukocyte antigen; FACS, fluorescence activated cell sorting; FITC, fluoresceinisothiocyanat; MHC, major histocompatibility complex; NT-3, neurotrophin-3; PE, phycoerythrin; CNS, central nervous system; INFγ, interferon γ; IS, immune system; PBMC, mononuclear cells of the peripheral blood.

* Corresponding author at: Immunotherapy Research Center IMMUNIS e.V., Pettenkoferstr. 8, D-80336 Muenchen, Germany. Tel.: +49 89 3090737 10; fax: +49 89 3090737 11.
E-mail address: professor-wank@muenchen-mail.de (R. Wank).
[1] These authors contributed equally to the work.

0171-2985/$ – see front matter © 2012 Published by Elsevier GmbH.
http://dx.doi.org/10.1016/j.imbio.2012.08.275

Patients and methods

Patients and control individuals

Patients ($n = 77$) and healthy random controls ($n = 214$) were German Caucasians.

Patients gave informed consent for us to use the obtained information for publication in accordance with the Declaration of Helsinki, including updates from Tokyo 1995, Hong Kong 1989, Sommerset West 1996 and Edinburgh-Scotland 2000. Schizophrenia was diagnosed according to the "Diagnostic and Statistical Manual of Mental Disorders" (DSM-IV) at the Psychiatric Hospital of the University of Munich.

Serological and molecular HLA typing

HLA class I typing

PBMCs were isolated from venous blood samples by Ficoll gradient centrifugation as previously described.

Serological HLA class I typing was performed using the Terasaki microdroplet assay (Terasaki et al., 1978). HLA-A was additionally typed by the Dynal AllSet + TM SSP HLA-A "low resolution" kit (Dynal Biotech, Germany).

HLA-J sequencing methods

The situation according to the new nomenclature is the following:

For *HLA-J M80468*:

*HLA-J*01:01:01:07* and *HLA-J*01:01:01:08* were earlier identified as *HLA-J M80468* and differ in exon 2 by one base exchange and in intron 6 by one base pair insertion. We now established a sequencing method to discriminate those two alleles. All of the 64 investigated control persons and 9 patients carrying *HLA-J 80468* were now found to be positive for *HLA-J*01:01:01:08*. (Three of the control samples could not be investigated by sequencing due to a lack of material.)

For *HLA-J M80469*:

Four of the nine newly defined alleles could not be found in our panel, as verified by reexamining our data. *HLA-J*01:01:01:01* and *HLA-J*01:01:01:02*, two of the remaining five alleles, differ only in one single nucleotide polymorphism (SNP) located in the intron 2 sequences and belong to the group *HLA-J M80469*. Because the SNP is located in an intron we combine these two alleles as *HLA-J M80469*.

For *HLA-J M 80470*:

HLA-J M 80470 is identical to HLA-J*02:01.

The division of the three formerly defined alleles into new alleles did not affect our results nor the significance of our results.

Genomic DNA was prepared using the proteinase K method. *HLA-J* exon 4 and exon 5 were sequenced using following primers:

PCR primers for *HLA-J* exon 4:

5′ Bio-AAGTGCCTGAGTTTTCCGAC 3′; 5′ TGAGGGTTCTGACCTCCAG 3′, Sequencing primer for *HLA-J* exon4: 5′ CY5-TGAGGGTTCTGACCTCCAG 3′.

PCR primers for *HLA-J* exon5:

5′ Bio-CTGGAGGTCAGAACCCTCA 3′; 5′ ACACATTTCTACCTGGAGCTT 3′, sequencing primer for *HLA-J* exon 5: 5′ CY5-AGTGGGACAAGAAAACTCAA 3′. Amplified PCR products were sequenced following strand separation with streptavidin-coated Dynabeads M-280 (Dynal Biotech, Germany). Sequencing was performed using Cy5 labeled primers; gels were run on an automated DNA sequencer (ALF Express, Pharmacia, Germany).

To distinguish *HLA-J*01:01:01:07* and *HLA-J*01:01:01:08* alleles which were identified as *HLA-J M80468* by the earlier nomenclature system, we designed the following primers:

PCR primers for HLA-J, intron 6:

5′GGCAGTTGGTCCAGGACCCACATCTG 3′, 5′ CATCAGAGCCCTGGGCACTTTGGCTGC 3′.

Sequencing was performed by the Sequencing Service Department Biology, 82152 Martinsried, using the following primer:

5′ ACAGAACCTGGTCAGATCCCACAG 3′.

HLA-J M80469 is identified by the new nomenclature system as *HLA-J*01:01:01:01:01:01 and HLA-J *01:01:01:01:01:02*, which only differ from each other by one base pair in intron 7. Since this difference has no influence on our conclusions, we did not sequence the samples carrying *HLA-J M80469*. *HLA-J M80470* is identical to *HLA-J*02:01*.

For simplicity we continued to use the designations *HLA-J M80468*, *HLA-J M80469*, *HLA-J M80470.*

FACS analysis

The PBMCs of patients and control individuals isolated by Ficoll gradient centrifugation were cultured at a concentration of 1×10^6 cells/ml at 37 °C in 2 ml RPMI 1640 supplemented with 10% FCS with (treated) or without (untreated) 100 U/ml recombinant human IFNγ-1b (Boehringer Ingelheim, Germany). After 16 h of incubation, PBMCs were harvested for FACS analysis. The PBMCs were subsequently stained with mouse biotin-anti-HLA-A25, 26(A10); anti-HLA-A2 (Acris GmbH, Germany); anti-HLA-A23, 24(A9) (One Lambda, Germany); or mouse monoclonal anti-HLA-A3 (kindly provided by Dr. D.J. Schendel). Cells were then counter-stained with FITC (fluoresceinisothiocyanat) labeled goat anti mouse IgG-IgM (for HLA-A2 and HLA-A3 study, Becton-Dickinson, Germany) or FITC-streptavidin (for HLA-A25, 26 study, Becton-Dickinson, Germany) and PE (phycoerythrin) labeled mouse anti human CD14 antibody (Becton-Dickinson, Germany). Samples were analyzed on a Becton-Dickinson FACSCan analyzer running CellQuest software (Becton-Dickinson, Germany). A total of 5000 CD14+ monocytes were collected to analyze HLA-A expression. Because different antibodies were used for HLA-A2, A3 and A10 (A25, 26), the relative mean fluorescence intensity (MFI) was used to compare the inducibilty of HLA-A expression by IFNγ, and the MFI was calculated as follows: relative MFI (%) = (treated MFI-untreated MFI)/untreated MFI × 100.

Statistical analysis

The statistical significance of frequency differences between patients and controls were calculated by X^2-tests; magnitude and statistical significance were determined by relative risk and 95% confidence interval (CI). A two-sided exact p value and, for expected frequencies smaller than 5, two-sided Fisher's exact p values were calculated.

The Mann–Whitney test was applied between groups of the same genetic group (patients and controls), and two-tailed exact p values were used. All statistical analysis was performed with the statistical program SPSS (v.10.1, SPSS Inc.).Fig. 1 was created with Microsoft® PowerPoint® 2000 (9.0.2716).

Results

Statistical association analysis of HLA-J/HLA-A combinations with schizophrenia

We sequenced exons four and five of the *HLA-J* gene in German Caucasian schizophrenic patients ($N = 77$) and in unrelated control individuals ($N = 214$) using still available material and cells from previously investigated patients (Laumbacher et al. 2003) (Table 1). Furthermore, we looked at the recently defined "splits" of *HLA-J M80468*. By sequencing we found that 64 of 67 control individuals