LETTER TO JMG

Significant frequency deviation of the class I polymorphism HLA-A10 in schizophrenic patients

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T he central nervous system (CNS) and the immune system communicate bidirectionally.[1][2] Immune cell subpopulations produce neurotrophins like neurotrophin-3 (NT-3), brain derived neurotrophic factor (BDNF), and dopamine.[3][4] Such subpopulations are vulnerable to infection and may impair feedback loops to neuronal cells as chronically infected carriers. Successful elimination of infection depends mainly on successful presentation of microbial antigens by HLA molecules, that is, on inherited HLA alleles. The recognition of a major contribution of genetic factors to the aetiology of schizophrenia marks one of the highlights in the research into schizophrenia. Foster parents of schizophrenics do not induce the disease in adopted children from healthy parents and healthy foster parents cannot prevent the development of schizophrenia in children from schizophrenic parents.[5] Different chromosomes have been pinpointed as harbouring genes involved in the pathogenesis of schizophrenia.[6][7] Several researchers have found evidence for schizophrenia vulnerability genes on chromosome 6p close to the HLA genetic region by linkage analysis.[8–11] The HLA region spans 4000 kb and contains more than 100 genes. The peculiarity of this region is the high degree of polymorphism of most loci, that is, these loci encode a multitude of alleles which have evolved to fight a multitude of microbial factors.

We analysed frequency deviations of HLA polymorphisms in two panels of patients with schizophrenia, collected at different times. HLA-A and HLA-C antigens were defined serologically in the patient and in random controls . HLA-C alleles were identified in addition by sequence based typing (SBT) using previously designed primers in the random controls and in patient panel 2.[12] Genetic typing of HLA-C was indicated because of a generally poor expression of HLA-C antigens and therefore difficult serological assessment. Furthermore, higher resolution of HLA-C by sequencing did not show any stronger association with schizophrenia than serological antigen typing. HLA-A antigens are generally well expressed and do not pose problems for serological typing of public determinants. Therefore, the HLA-A polymorphism was investigated only serologically. We were interested in the HLA-A locus because it could give a lead to further investigations of closely linked genes like HLA-G involved in the protection of the trophoblast.

PATIENTS AND METHODS
Patients and random controls
All patients were white and from southern Germany. The diagnosis of schizophrenia was performed at the Psychiatric Hospital of the University of Munich. Schizophrenia was diagnosed according to the third edition of the "Diagnostic and Statistical Manual of Mental Disorders" (DSM-III-R) in patients in panel 1 and panel 2. All patients were inpatients at the time of diagnosis. Different schizophrenic subtypes had no impact on the observed deviation of frequencies of HLA class I alleles and antigens (not shown).

Patient panel 1 (n=31) was previously collected to study HLA class I antigens and HLA class II alleles. Results of HLA

Key points

- We compared frequencies of HLA-A and HLA-C antigens in two patient panels with schizophrenia, panel 1 (n=31 for both) and panel 2 (n=60 and n=71, respectively) and a random panel (n=367 and n=390, respectively) and found a lower frequency of HLA-Cw5 in both patient panels, which did not reach significance.

- The increase in frequency of HLA-A10 was nearly identical in both patient panels (p=0.006, two sided, in panel 2, relative risk 3.071). Association of other genes in the HLA-A chromosomal region with schizophrenia will be discussed.

class II alleles have already been published.[13] Patient panel 2 (n=71) was collected later over a three year period and HLA-C polymorphism was investigated by sequence based typing (SBT) for best resolution. Only 60 of these 71 patients could be used for serological typing.

Samples for the random control panels were collected from members of the Institute of Immunology in Munich and from medical students at the University of Munich. All subjects were white. The frequencies of HLA antigens did not deviate from other published German HLA antigen frequencies.

Isolation of peripheral mononuclear cells (PBMC), RNA, and HLA typing
Peripheral blood mononuclear cells (PBMC) from healthy random controls and schizophrenic patients were isolated by Ficoll gradient centrifugation.

Serological HLA-A typing was performed according to the NIH method with some modifications to the original technique.[14] Our own monoclonal antibodies, reagents obtained by exchange, and commercial reagents were applied. A total of 28 HLA-A and -C antigens were defined using a battery of 120 reagents. Total RNA was prepared from freshly isolated cells or after CD3 stimulation using the Micro RNA isolation kit (Stratagene, La Jolla, Ca 92037, USA). RNA was transcribed into cDNA by RAV-2 reverse transcriptase (Amersham, Braunschweig, Germany) and amplified by PCR. Sequence, length, and localisation of primers were as previously described.[12] Sequencing primers were hybridised to conservative regions of HLA alleles. Amplified PCR products were sequenced after strand separation with streptavidin coated Dynabeads M-280 (Dynal, Hamburg, Germany). Sequencing was performed using fluoroprime or indodicabo-cyanine labelled primers and gels were run on an automated DNA sequencer (ALF Express, Pharmacia, Freiburg, Germany). Both strands of every PCR product were sequenced. Twenty-three different HLA-C alleles were identified in patients and controls (not shown).

Statistical evaluation
HLA-A and HLA-Cw antigen frequencies of patients and random controls as well as HLA-Cw* allelic frequencies (not

Table 1 Phenotype frequency of HLA-Cw5 and HLA-A10 in schizophrenic patients

	Normal controls	Schizophrenic patients	
		Panel 1	Panel 2
(A) Phenotype frequency of HLA-Cw5 in schizophrenic patients			
	(n=390)	(n=31)	(n=71)
HLA-Cw5	16.65%	9.67%	9.85%
Chi-square (Pearson)		1.03	2.11
Fisher's exact p, two sided		0.44	0.16
(B) Phenotype frequency of HLA-A10 in schizophrenic patients			
	(n=367)	(n=31)	(n=60)
HLA-A10	6.81%	19.35%	18.33%
Chi-square (Pearson)		6.26	8.86*
Exact significance, two sided p		0.025	0.006

*95% confidence interval 1.422 to 6.631, relative risk 3.071.

shown) were compared using Pearson χ^2 statistics with 95% confidence intervals; exact significance p values were determined two tailed (table 1). Uncorrected significant p values were confirmed in HLA-A10 in panel 2. Therefore, the p value of HLA-A10 did not require correction by investigated antigens. Data were calculated using the statistical programme SPSS (version 10.1.3, SPSS Inc).

RESULTS

Both patient panels and controls were typed for 20 HLA-A antigens and eight HLA-C antigens serologically. The following HLA-C alleles were identified by sequence based typing: HLA-Cw*0102, -Cw*02022, -Cw*0302, -Cw*0303, -Cw*0304, -Cw*0401, -Cw*0501, -Cw*0602, -Cw*0701, -Cw*0702, -Cw*0704, -Cw*0802, -Cw*1202, -Cw*1203, -Cw*1502, -Cw*1504, -Cw*1505, and -Cw*1601. The random control panel is not shown because the results of HLA-C typing of a major part of the control panel has previously been published.[12] As shown there, HLA-Cw5 was encoded only by one allele, HLA-Cw*0501. Nine patients were identified as carrying HLA-Cw*0501 and these patients also expressed HLA-Cw5 serologically. We did not find a significant difference between HLA-SBT typing and serological typing for HLA-Cw 5/HLA-Cw*0501. No significant HLA-C allelic frequency deviation was found in patient panel 2 as compared to the random controls (not shown), nor of HLA-Cw4 as previously published.[15] Therefore the results were restricted to serological typings of patient panel 1 and patient panel 2 (table 1A). From 20 serologically tested HLA-A antigens (not shown), only HLA-A10 showed a significant frequency deviation. The

results of HLA-A10 in patient panel 1 (p=0.025, corrected, not significant) was replicated in patient panel 2 (p=0.006, p value correction not required, legend table 1)

DISCUSSION

Many microbial factors have been implicated in the pathogenesis of schizophrenia, but so far each microbial factor has been identified in a relatively small subgroup of patients.[16–20] The heterogeneity of these microbial factors is also reflected by the associations with different HLA loci and their alleles. Polymorphic HLA alleles encode individual smell receptors[21] and influence mating preferences.[22] Polymorphic HLA molecules process, select, and present degraded microbial proteins.[23 24] The set of inherited HLA alleles determines susceptibility or resistance to particular microbes.

We recently analysed immune response genes of the HLA class II region in patient panel 1 with sequence specific oligonucleotide typing and reported an increased frequency of the narcolepsy and multiple sclerosis associated HLA class II allele DQB1*0602 in the schizophrenic patients.[13] A former report of a strongly increased frequency of HLA-Cw4 in Czech schizophrenic patients[15] inspired us to reinvestigate HLA-C polymorphism by sequencing the schizophrenia patient panel 2 (not shown). However, we did not find a significantly increased frequency of any HLA-Cw allele in the patients, rather a decreased frequency of the allele HLA-Cw*0501 (not shown). This allele is the only one to encode the serologically defined antigen HLA-Cw5.[12] Although the results of patient panels 1 and 2 showed a similarly decreased frequency of HLA-Cw5, the decrease in frequency did not reach significance
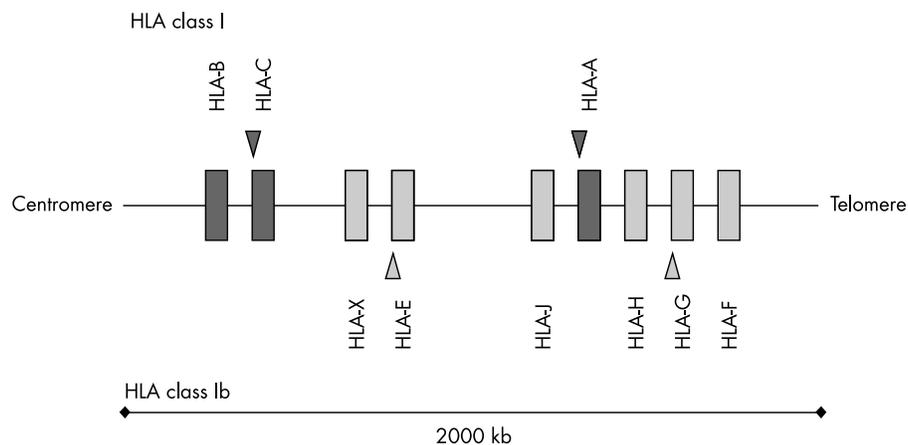

Figure 1 Localisation of discussed non-classical class I genes within the HLA class I region. HLA-B, -C, and -A encode the classical class I MHC molecules. HLA-E, -F, and -G represent non-classical class I genes encoding HLA class Ib molecules. HLA-X, -J, and -H are at present categorised as pseudogenes.

(table 1A). The significant serologically defined increased frequency of HLA-A10 in patient panel 1 (p=0.025, table 1A) caused us to look at the frequency of HLA-A10 in patient panel 2. We found a nearly identical increased frequency of HLA-A10 in patient panel 2 (table 1B).

It is worth noting that higher resolution of antigenic determinants, either by HLA-Cw sequencing or further subdivision of the HLA-A10 antigen (not shown), did not strengthen the degree of association with schizophrenia. This could indicate that several different microbial factors were presented by this public HLA-A10 antigen, but we favour a lesser association of HLA-A10 with schizophrenia because of genes in the chromosomal vicinity of the HLA-A locus. Two loci in the close neighbourhood of HLA-A (fig 1) appear to be very good candidates for further investigations, HLA-G and HLA-E. Both genes are polymorphic and play an important role during embryogenesis. They are also expressed by activated immune cells in adults. Investigations of polymorphisms in this genetic region could be particularly interesting in mothers causing schizophrenic birth excesses, infected by influenza epidemics, or by Borrelia Burgdorferi.[15][25] Infected immune cell subpopulations may fail to protect embryonal brain development or fail to produce, for example, brain derived neurotrophic factor (BDNF).[3] Infections of such subpopulations in adults could modulate/disturb the neurotrophic system and thereby influence neuronal adaptive responses. Restoration of such circuits by adoptive immunotherapy may have led to the substantial improvement observed in three psychiatric patients, suffering from autism, bipolar depression, or schizophrenia.[26] It will be worthwhile to study HLA polymorphisms further in order to identify immunogenetic dispositions, that is, HLA alleles lacking resistance against certain microbial infections. It will be important to identify in these chronically infected patients immune cell subpopulations that function in communication with the central nervous system.

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