Viral Antibody Titers, Immunogenetic Markers, and Their Interrelations in Multiple Sclerosis Patients and Controls

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ABSTRACT: Our purpose was to investigate possible interrelations between antibody titers against seven viruses (measles, rubella, herpes simplex, mumps, varicella-zoster, coronavirus, cytomegalovirus), HLA-class II antigens, and immunoglobulin Gm allotypes in multiple sclerosis (MS). We studied 57 MS patients and 59 controls with similar age and sex distributions. In MS patients, we found the classical increased frequency of HLA-DR2, HLA-DQw1 and also an excess of Gm (3; ±23; 5*). Mumps antibody levels were higher in MS patients than in controls; elevation was not significant for measles antibodies. Analysis suggests that an association between HLA-DQw1 and antibody titers against various viruses exists in controls but is absent in MS patients. In particular, we found that mumps antibody titers were higher in DQw1-positive than in DQw1-negative controls, while there was no significant difference among MS cases. Accordingly, we found that the overall difference between patients and controls was due to the fact that DQw1-positive patients had higher titers than controls, while DQw1-negative cases had similar titers as controls. These findings suggest that biological and molecular characteristics of DQw1 might differ in MS patients. *Human Immunology 31, 94–99 (1991)*

ABBREVIATIONS

Gm   heavy chain immunoglobin G   MS   multiple sclerosis

INTRODUCTION

The cause of multiple sclerosis (MS) is complex and many facts observed repeatedly during the two last decades still remain unexplained. Elevated antibody titers are found in serum and cerebrospinal fluid of patients with MS, against measles, and less frequently against other viruses including herpes simplex, varicella-zoster, rubella, vaccinia, influenza, and Epstein-Barr viruses [1–3]. These findings can be interpreted in various ways. Elevated antibody titers may be either a consequence of the disease itself, or in relation with the role of various common viruses in the pathogenesis of MS. Another explanation is that increased levels of viral anti-bodies in MS patients may be related to immunogenetic factors associated with MS. Thus, because of specific structural complementarities of antigens, immune response against viral antigens might be controlled by genes linked with the HLA alleles associated with MS. This hypothesis is supported by several reports on the difference in response to vaccines (poliomyelitis, diphtheria typhoid, vaccinia virus) and viral antigens (rubella, measles, influenze, and Epstein-Barr viruses) in relation with HLA phenotypes [4–7], especially with HLA-DR antigens. Antibody levels after immunization against various infectious agents depend also on genes coding for immunoglobulin G heavy chains (Gm) [8], and association between Gm allotypes and susceptibility to MS has been reported [9,10].

The purpose of our study was to investigate possible interrelations between viral antibody titres, HLA-class II antigens and immunoglobulin Gm allotypes in MS patients and controls.

MATERIALS AND METHODS

The present investigation is a part of a population-based case-control study on MS which was conducted in the Hautes-Pyrénées "département" (a jurisdiction in the southwest of France). Ascertainment of MS cases is described with details elsewhere [11]. Only patients with definite or probable MS according to the criteria of Poser et al [12] were included. A control group consisting of healthy individuals of similar age and sex as the patients and living in the same area was selected for comparisons. The geographical origin of patients and controls was documented by their birthplace and that of their parents.

The present investigation was restricted to the major histocompatibility complex class II antigens, HLA-DR 1-w14 and HLA-DQ w1-w3. Typing was performed by the two-color fluorescence method using 49 anti HLA-DR and 9 HLA-DQ alloantisera or monoclonal antibodies, either local or obtained by exchanges following the Eighth and Ninth Histocompatibility Workshops.

Samples were also tested for Gm allotypes of IgG1: G1m (1, 2, 3, 17), IgG2: G2m (23), and IgG3: Gm (5, 10, 11, 13, 14, 15, 16, 21, 28). Allotyping was performed by the classical hemagglutination inhibition method on opaline plates at room temperature [13]. The reagents used for determination were of human origin, except for the G2m [23] allotype (rabbit antisera). The allotypes IgG3 (5, 10, 11, 13, 14) are designated by 5*.

Rubella, measles, cytomegalovirus, herpes simplex type 1 virus, varicella, and mumps antibodies were determined using ELISA technique (Enzygnost Behring). Titers were defined by comparing optic density between each sample and serial dilutions of a known reference serum. Log-logit regression curves of this reference serum were established on computer.

Coronavirus antigen was prepared in our laboratory from bovine enteric coronavirus (BECV) (stain C_3II100) cultivated on HRT 18 (human rectal adenocarcinoma), kindly supplied by J. Laporte (INRA, Grignon, France). HRT 18 cells were grown in RPMI 1640 medium supplemented with 20% fetal bovine serum, 100 u/ml penicillin, and 0.1 mg/ml streptomycin. Two-day-old confluent monolayers were inoculated with BECV diluted in RPMI 1640 supplemented with 2% fetal bovine serum and antibiotics. The inoculum was allowed to adsorb for 1 hr at 37°C with manual rocking every 10 min, then the medium was removed and fresh medium added.

After an incubation period of 3 days at 37°C, cell cultures were frozen and thawed; suspension was homogenized and centrifuged at 3400 g for 10 min. The supernatant was then centrifugated at 18,500 g for 1 hr (Ultra centrifuge, Beckman, Rotor 50Ti). The supernatant was removed and the pellet homogenized in buffer pH 9.6 (Na_2CO_3 0.1 M, $NaHCO_3$ 0.1 M). After centrifugation for 10 min at 3400 g, the supernatant was titrated by checkboard technique using human serum with high titer of coronavirus antibodies. The same, previously described, technique of ELISA titration was used for the determination of coronavirus antibodies.

All titers were compared by transforming to log 2 the reciprocal of dilutions (geometric titer). An arbitrarily small number was assigned to trace and undetectable titers.

All typings and serological measurements were performed blindly.

Statistics. Antigen frequencies were compared by the χ^2 test and geometric titers by the two-tailed *t*-test. Variance analysis was performed for relationships between genetic marker frequencies and viral antibody titers.

RESULTS

Patient population. We studied 57 MS patients: 42 females and 15 males. The mean age at the time of the study was 43.9 yr. There were 35 patients with a relapsing-remitting form of MS and 22 patients with a primary or secondary chronic progressive course of the disease. Controls (*n* = 59) had similar mean age (45.7 yr) and sex ratio as MS patients. Forty-eight patients (84%) and all controls originated from the southwest of France or from Spain.

For technical reasons, some data was missing but neither the size of the MS patient group nor that of the control group was ever lower than 53.

Antibody titers in MS patients and controls. Mumps antibody titers were significantly higher in MS cases than in controls; mean geometric titers were 6.31 and 5.66, respectively (*p* = 0.002). As shown in Table 1, patients and controls had comparable antibody titers against the six other viruses. Antibody titers against measles were slightly higher in MS patients than in controls but the difference was not statistically significant.

HLA-DR, HLA-DQ, and Gm antigens in MS patients and controls. Of the DR-locus antigens, HLA-DR2 was present in 54.3% of MS patients and 23.7% of controls (*p* = 0.001). The frequency of HLA-DQw1 was also higher in patients than in controls (80.7% vs. 54.2%, *p* = 0.01). No difference was found for any of the other HLA-DR and HLA-DQ antigens.

The detailed results of Gm allotype frequencies in patients and controls have been reported [9]. A significant excess of patients with the phenotype Gm (3; ±23;

TABLE 1   Viral antibody titers in MS patients and controls

| | Geometric titer | | | |
| | MS patients | | Controls | |
Virus	Mean	SD	Mean	SD
Herpes simplex	6.60	1.40	6.58	1.27
Measles	7.41	1.10	7.10	1.18
Varicella-zoster	6.37	1.17	6.52	1.06
Mumps	6.31	1.22	5.66[a]	1.10
Rubella	6.17	1.13	6.29	1.03
Cytomegalovirus	5.68	1.43	5.44	1.23
Coronavirus	6.77	1.27	6.99	1.24

[a] $p = 0.002$.

5*) was observed: this phenotype was present in 59.6% of MS patients and in 37.2% of controls ($p = 0.02$).

The frequencies of these genetic markers were similar among relapsing-remitting and chronic progressive patients: 51.4% vs. 59.1% for DR2, 82.8% vs. 77.3% for DQw1, and 57.1% vs. 63.6% for Gm (3; ±23; 5*), respectively.

Relationship of viral antibody titers with HLA-DR2, HLA-DQw1, and Gm (3, ±23; 5) in MS patients and controls.* Relationships between viral antibody titers and the genetic markers associated with MS were analyzed separately in MS patients and in controls.

The first result of this analysis was that antibody levels in controls correlated with the presence of DQw1 (Table 2). This correlation was either positive or negative according to the virus. Thus, antibody titers against measles, rubella, and coronavirus were significantly higher in DQw1-positive than in DQw1-negative controls, while antibody titers against cytomegalovirus were significantly elevated in DQw1-negative controls. No general pattern of association between DQw1 and viral antibody titers was observed in MS patients. Interestingly, only mumps antibody titers differed significantly according to whether MS patients were DQw1-positive or DQw1-negative.

The two other markers, DR2 and Gm (3, ±23; 5*) (Table 2), did not appear to be related with viral antibody titers, neither in controls nor in MS patients. We found new significant results which might be interpreted as chance results. Thus, DR2-positive controls had higher levels of antibodies against coronavirus than DR2-negative controls. The phenotype Gm (3, ±23; 5*) was significantly associated with higher titers against cytomegalovirus in controls and lower titers against varicella-zoster in MS patients. But on the whole, viral antibody levels were very similar for all Gm phenotypes.

The second step of this part of the analysis was to compare antibody titers in MS patients and controls within six subgroups with identical HLA-DR2 (+/−), HLA-DQw1 (+/−), or Gm (3, ±23; 5*) (+/−) phenotypes.

Overall, this immunogenetic adjustment did not re-

TABLE 2   Differences of mean antibody titers according to the presence or absence of genetic markers in MS patients and in controls[a]

| | DQw1 | | DR2 | | Gm (3; +23; 5*) | |
	MS	Controls	MS	Controls	MS	Controls
Antigen present	46	32	31	14	34	22
Antigen absent	11	27	26	45	23	37
Mean titer differences	0.08	0.72[b]	0.13	0.32	0.02	0.05
Measles	0.63	0.31	0.41	0.40	0.82[c]	0.11
Varicella-zoster	0.32	0.66[b]	0.50	0.02	0.02	0.25
Rubella	0.85[b]	0.10	0.48	0.24	0.30	0.19
Mumps	0.18	0.87[c]	0.13	0.53	0.33	0.63[b]
Cytomegalovirus	0.32	0.65[b]	0.33	0.95[c]	0.0	0.06
Coronavirus						

[a] Differences are positive when mean geometric titers are higher, when the marker is present. A rough correspondence between differences(d) and ratios(r) of titers can be estimated as follows: d = 0.10 ≈ r = 1, d = 0.30 ≈ r = 1.3, d = 0.50 ≈ r = 1.6, d = 0.70 ≈ r = 2, d = 0.90 ≈ r = 2.5. A difference of 0.70 means than titers are approximately 2.0 times higher in positive than in negative individuals; a difference of −0.80 means that titers are approximately 2.3 times lower in positive than in negative individuals.
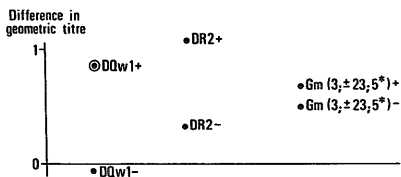[b] $p < 0.05$.
[c] $p < 0.01$.

FIGURE 1 Differences in mumps antibody titers in MS patients and controls matched on HLA-DR2, HLA-DQw1, and Gm (3; ±23; 5*). Points above the zero line when titers are higher in patients than in controls. Significant differences are encircled.

veal any significant differences between MS patients and controls, except for mumps. Points on Fig. 1 represent the differences of mean mumps antibody titers between MS patients and controls, in the six subgroups; points are above the zero line when titers are higher in patients than in controls. Multiple sclerosis patients who were positive for DQw1 had significantly higher levels of antibodies against mumps than DQw1-positive controls, while mumps antibody titers were similar in DQw1-negative patients and DQw1-negative controls. Also, DR2-positive MS patients had higher titers than DR2-positive controls, but this difference was not statistically significant. In the two subgroups defined according to Gm (3, ±23; 5*) phenotype, antibody titers were higher in MS patients than in controls (Fig. 1).

DISCUSSION

Besides the classical association of MS with HLA-DR2 and HLA-DQw1, we found an increased frequency of Gm (3, ±23; 5*) in MS patients who lived in the Hautes-Pyrénées by comparison with controls living in the same area. Antibody titers against seven viruses were measured and anti-mumps antibody levels were significantly higher in MS patients than in controls. No significant difference was found for measles; however, MS patients had slightly higher titers than controls.

Association between antibody titers and immunogenetic markers was analyzed. The first important observation was that viral antibody titers in control individuals varied significantly depending on the presence or absence of DQw1. The presence of DQw1 antigen was associated with elevated antibody titers against measles, rubella, and coronavirus and low antibody titers against cytomegalovirus. Differences in the basic biological and molecular properties of viruses may explain that correlation between HLA antigens and viral antibody titers

are either positive or negative. Conversely, as it is now well known that peptides of antigens are associated to HLA-class II molecules in order to be presented to T cells, affinity of virus peptides could vary with different HLA-class II molecules, giving rise to variable immune responses. This has been shown for influenza matrix peptide [14].

Correlations between various HLA antigens, and especially class II antigens, and antibody titers have been reported in normal individuals. Kato et al. [6] suggested that the gene(s) controlling the low responsiveness to rubella might be located in the sixth chromosome, near the HLA-DR locus. Cohen et al. [7] observed that individuals with HLA-DR1 or HLA-DR5 had significantly higher antibody titers against Epstein-Barr virus capsid and nuclear antigens than those without DR5 or DR1. These studies have been done before HLA-DQw1 was identified.

Multiple sclerosis patients differed from controls. In the MS group, viral antibody titers were not related to DQw1, with a remarkable exception for mumps. Therefore, our study suggests that an association between HLA-DQw1 and antibody titers against various viruses does exist in normal individuals but is absent in MS patients.

Most of the previous studies on the relationship between HLA antigens and viral antibody titers in MS patients have focused on measles virus. Following the study by Jersild et al. [15], which showed an association between high measles antibody titers and HLA-A3, HLA-B7, and HLA-B18 antigens, several negative reports have been published [16–18]. No relationship was noted between HLA type and measles antibody titers within families with several affected individuals [19]. Poskanser et al. [20] correlated HLA phenotypes with antibodies directed against eight viruses in patients with MS and found no association. These studies have been done in the 1970s when HLA-DR antigens had not yet been defined. To our knowledge, there are no published results on interrelationships between class II antigens and viral antibody titers in MS. But it may be noted that interrelationships between HLA-class II antigens and viral antibody titers have been found in insulin-dependent diabetes [21] and alcoholic cirrhosis [22].

A clear explanation of our findings is not easy. It may suggest that MS patients have an immunological dysfunction which interferes with the normal control of immune response by genes belonging to or linked with the HLA region. The most attractive hypothesis is that biological and molecular characteristics of DQw1 might differ in MS patients. Gene(s) nonlinked to DQ but influencing its function, such as those controlling molecules that are involved in cellular adhesion between T

lymphocytes and antigen-presenting cells, could also play a role in the modification of viral immune response in MS [23].

A major finding of this study was observed when comparing mumps antibody titers in MS cases and controls. We found that the difference between all MS cases and all controls could be attributed to the difference between all DQw1-positive cases and controls. In contrast, mumps antibodies titers were similar in DQw1-negative MS patients and controls.

Some studies support a possible role of mumps virus in MS. Local synthesis in the central nervous system of oligoclonal antibodies against mumps and other viruses with neurotropic properties has been observed [24]. Several studies including multiple comparisons of viral antibody titers in MS patients and controls have been done and higher titers of mumps antibodies in MS cases than in controls have been reported in some of them [25]. Significant associations may occur by chance and must be interpreted with caution. However, the fact that elevated antibody titers against mumps in MS patients depended on the presence or absence of DQw1, an HLA antigen associated with susceptibility to MS in our population, gives some consistency to our finding. It raises the question as to whether differences in mumps antibody titers between MS patients and controls might be due to particular DQw1 characteristics present in these patients, particularly characteristics that might play a role in susceptibility to MS.

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