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Serological differences in monozygotic twin pairs discordant for multiple sclerosis

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Objectives – The etiology of MS is unknown but genetic factors are supported by a high concordance in twins. Geographic distribution and migration studies indicate, however, the importance of environmental factors. **Material and methods** – We studied 3 pairs of genetically identical twins who had shared the same environment but were discordant for MS. Serum samples were assayed for antibodies against 21 viruses, 4 bacteria and *Toxoplasma gondii*. **Results and conclusion** – No common factor present only in the affected twins was identified but differences were found in serum titers against some neurotropic microorganisms. In general the serum titers were strikingly similar in the twins, indicating no major disturbances of the humoral immune system in MS.

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Key words: multiple sclerosis; twins; virus; antibodies

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Multiple sclerosis (MS) is a common demyelinating disorder of the CNS in young adults. In Scandinavia the prevalence is approximately 1%. The etiology of the disease is unknown but an autoimmune component and genetic factors are believed to be involved (1, 2). On the other hand, geographic distribution, data from migration studies and the fact that more than 70% of monozygotic twins are discordant for MS, indicate that environmental factors are important. The aim of the present study was to search for environmental differences that could be important for the development of MS in 3 pairs of genetically identical twins discordant for MS.

Material and methods

Three pairs of twins, 2 male pairs and 1 female pair, were investigated. Monozygosity was confirmed by the use of minisatellite analysis giving a negligible error of 0.0001. The twins were born in 1970, 1975 and 1976. One twin in each pair had clinically definite MS by standard criteria (3). The diagnosis was supported by typical MRI lesions and oligoclonal Ig bands in the spinal fluid. Onset of MS was between 1 and 7 years before the investigation started.

Discordance in the healthy twins was based on medical history and clinical examination and in one case supported by a normal MRI.

Medical and social histories were obtained by questionnaires including infectious and other diseases, allergies, vaccinations, exposure to animals, smoking habits, occupational and chemical exposures, and travelling in foreign countries.

Serum samples were obtained and assayed for antibodies against 21 viruses, 4 bacteria and *Toxoplasma gondii*. Immunoassay was used for influenza A and B, adenovirus, RS-virus, parainfluenza virus 1, 2 and 3, *Mycoplasma pneumoniae*, herpes simplex and varicella zoster virus, cytomegalovirus, EB-virus EBNA, mumps, morbilli, enterovirus, parvovirus, rubella, HHV-6, *Borrelia burgdorferi*, *T. gondii*, *Chlamydia trachomatis*, *Chlamydia pneumoniae* and *Chlamydia psittaci*. Antibodies against poliomyelitis virus 1, 2 and 3 were studied employing neutralization tests. Commercially available AxSym (Abbott) was used to detect HbsAg, anti-HAV, anti-HCV and anti-HIV1/2, EIA (Abbott) for HIV1 Ag and EIA (Murex) for anti-HTLV1/II.

The investigation was approved by the Ethics Committee. All 6 individuals gave informed consent.

Antibody titers in twins discordant for MS

Table 1. Swedish national vaccination program

Age	Vaccination
3 months	DTP I (diphtheria-tetanus-pertussis) + polio I + Hib I (H. influenzae)
5 months	DTP II + polio II + Hib II
12 months	DTP III + polio III + Hib III
18 months	measles-mumps-rubella I
5-6 years	polio IV
10 years	DT (diphtheria-tetanus) IV
12 years	measles-mumps-rubella II

Results

Twin pair No. 1

The affected twin noted his first MS symptoms (double vision, imbalance and sensory disturbance) at the age of 28 and MS was diagnosed in the same year. The parents, an older brother and the twin brother are healthy. The twins lived together until the age of 20. They kept pet guinea pigs and birds. Both started to smoke at 15 and both were still smokers at the time of the investigation. At age 21 the unaffected twin went through an ordinary military service but the twin who would later develop MS underwent a strenuous training period in the coastguard. Both have an academic education. They have not been working in hazardous environments or been exposed to toxic substances. Both received the usual immunizations during childhood (Table 1), including vaccination against polio. The unaffected twin had recurrent episodes of tonsillitis and suffers from cold hyperreactivity. The MS-affected twin had mononucleosis at the age of 15, borreliosis at 21 and pneumonia at the age of 22. He had no infection in the period immediately preceding the onset of MS. The affected twin had no neutralizing antibodies against polio type 3 and low titers against types 1 and 2 (Table 2). After this study he was revaccinated which resulted in a normal antibody response (data not shown). He also had a borderline IgM serum antibody titer

against *B. burgdorferi* that probably could be explained by the Borrelia infection in 1992.

Twin pair No. 2

The affected twin noted her first neurological symptom (vertigo) at the age of 20 and MS was diagnosed 2 years later. The parents, a younger sister and the twin sister are healthy. These female twins were still living together at the time of the investigation. During childhood they kept a pet guinea pig and after the age of 14 they have had a cat. They have never smoked. At 19, both worked for 1 year as au-pair girls in the US and lived with different families. The twins have not been working in hazardous environments or been exposed to toxic substances. Both received usual immunizations during childhood, including mumps. Both girls had chickenpox at the age of 13. The twin who would later develop MS had pertussis at 5. At 13 she suffered a hemorrhage in her right eye with a permanent loss of vision. The condition was diagnosed as a Coat's retinitis. She had no obvious infection in the period immediately prior to the onset of MS.

There were two serological differences between the twins (Table 2). The MS-affected twin had IgG antibodies against Toxoplasma but no IgG antibodies against mumps virus.

Twin pair No. 3

The affected twin noticed the first neurological symptoms (muscle spasms) at the age of 17 and MS was diagnosed 2 years later. The parents, 2 older brothers and the twin brother are healthy. These twin brothers were still living together at the time of the investigation. They kept no pet animals and have never smoked. Both are university students and have never worked in hazardous environments or been exposed to toxic substances. In the unaffected twin, a diagnosis of celiac disease was established when he was 1 year old. Both twins had chickenpox at age 7.

Table 2. Differences in antibody titers and antigens in three monozygotic twin pairs

Virus	Twin pair No. 1		Twin pair No. 2		Twin pair No. 3	
	MS-Affected twin	Unaffected twin	MS-Affected twin	Unaffected twin	MS-Affected twin	Unaffected twin
Mumps virus IgG	300	300	neg <100	100	1000	1000
Polio 1 neutr. antibodies	0.1 IU/ml	>4 IU/ml	>4 IU/ml	>4 IU/ml	>4 IU/ml	>4 IU/ml
Polio 2 neutr. antibodies	0.5 IU/ml	>8 IU/ml	>8 IU/ml	>8 IU/ml	>8 IU/ml	>8 IU/ml
Polio 3 neutr. antibodies	neg <0.2 IU/ml	>5 IU/ml	>5 IU/ml	>5 IU/ml	>5 IU/ml	>5 IU/ml
>5 IU/ml						
<i>B. burgdorferi</i> IgG	neg <0.9	neg <0.9	neg <0.9	neg <0.9	neg <0.9	neg <0.9
<i>B. burgdorferi</i> IgM	marginal value 1	neg <0.9	neg <0.9	neg <0.9	neg <0.9	neg <0.9
Protozoae						
Toxoplasma IgG	neg <6 kIU	neg <6 kIU	45.3 kIU	neg <6 kIU	neg <6 kIU	neg <6 kIU
Toxoplasma IgM	neg	neg	neg	neg	neg	neg

Although both received the usual immunizations during childhood, including mumps, they had mumps at the age of 8. The later MS-affected twin had no infection in the period immediately prior to the onset of MS.

In these twins there were no major differences in the serum analyses although minor differences in the titers were observed.

HLA typing

The 3 twin pairs were analyzed for HLA class I and HLA class II (DR). One of the twin pairs had the DRB1*15 allele which is known to be associated with MS (Table 3).

Discussion

Geographic distribution, data from migration studies and the fact that most monozygotic twins are discordant for MS, indicate that environmental factors contribute to the development of the disease. Monozygotic twins are ideal when searching for triggering events, since they are genetically identical. Therefore we investigated 3 monozygotic twin pairs discordant for MS. The unaffected individuals are young and will be at risk for many years but at the time of investigation they were all healthy with no clinical signs of MS. Thus, it is likely that they have not yet been exposed to any environmental factor that can trigger the onset of the disease.

Because of his worries one of the healthy twins asked for and was examined with MRI, which was normal. The other 2 healthy twins have not asked for this and MRI has not been performed for ethical reasons.

The twins shared the same environment during childhood and adolescence and the serological analyses therefore revealed very similar antibody patterns. There were, however, some differences in the antibody response to agents which exhibit neurotropic properties.

The MS-affected twin in pair 2 had an IgG titer against *T. gondii*. From the age of 14 the twins had a cat, a possible source of infection. Acquired toxoplasmosis may mimic MS (4). MRI lesions in MS and toxoplasmosis are distinct, however, and in this

patient the MRI lesions were typical for MS. *T. gondii* is also an important cause of ocular disease both in immunocompetent and immunosuppressed individuals (5). The MS-affected twin had a Coat's retinitis but there were no signs or pathological changes typical for toxoplasmosis.

One MS affected twin had a history of serologically verified *Borrelia* infection which was treated with antibiotics. He had a borderline IgM titer but no IgG antibodies. It has been discussed how to distinguish between *Borrelia* infections and MS since there are some similarities in symptoms and spinal fluid reactions. However, the disease caused by persistent *Borrelia* is distinct from MS (6,7). Only a small fraction of the MS patients have antibodies against *B. burgdorferi* and antibiotic treatment against *Borrelia* has no effect on subsequent MS-relapses (8).

There is no evidence that *Toxoplasma* or *Borrelia* causes MS in the patients but it cannot be excluded that these pathogens could have acted as precipitating factors.

The herpes viruses (herpes simplex, herpes zoster, HHV-6, CMV, EBV) have been implicated as precipitating agents in MS. Treatment with Valaciclovir has been shown to reduce both clinical and MRI disease activity in MS (9). In our 6 twins the antibodies to these viruses were similar in the affected and unaffected individuals.

In general the serum titers were strikingly similar in the MS-affected and non affected twins, indicating that MS is not associated with major disturbances of the humoral immune system. Compared to the population in general, 4 of the investigated twins had poor responses to the vaccination against mumps. All the twins were vaccinated against mumps (given as a triple together with measles and rubella) during childhood. Yet the twins in pair 2 had no or low antibody titers against mumps virus and both twins of pair 3 had clinical mumps despite previous vaccination. Their titers against measles and rubella were normal indicating that the twins really were vaccinated. This finding may indicate a genetically determined weakness in their responsiveness to mumps vaccinations. The significance of this observation is, however unclear since the mumps vaccine was reported to be less efficient (Dr T. Dalianis personal communication).

Taken together, our observations of poor antibody titers after vaccination against mumps and polio, may indicate an impaired responsiveness to these immunizations. All six twins had received repeated polio vaccinations as part of the common childhood immunization plan, yet 1 MS-affected twin had no neutralizing antibodies against polio type 3 and low levels against types 1 and 2. However

Table 3. HLA types in three monozygotic twin pairs

	Twin pair No. 1	Twin pair No. 2	Twin pair No. 3
HLA Class I A	Allele 24/68	Allele 1/3	Allele 1/24
HLA Class I B	53/57	7/7	8/40
HLA Class II DR	7/13	4/15	3/13

Antibody titers in twins discordant for MS

when he was revaccinated recently he responded with normal antibody titers showing that in this respect he has a fully functional humoral immune system. The reason for the previously low titers is not known. It is possible that he did not receive all 4 polio vaccinations as a child, or that other infections inhibited the immunization.

Our study in MS-discordant monozygotic twins did not identify a unique factor present only in the affected twins. A similar study including 8 Finnish monozygotic twin pairs also found no support for the view that MS was triggered by any of the investigated viruses (10).

Several common childhood infections have been proposed to be involved in MS. This hypothesis was not supported by the findings in our study. Furthermore the vaccination programs against these diseases have not yet changed the incidence of MS in young adults.

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