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Incidence of CSF abnormalities in siblings of multiple sclerosis patients and unrelated controls

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Abstract We found that 19 % (9/47) of healthy siblings of patients with clinically definite multiple sclerosis had an intrathecal immunological reaction with two or more 2 CSF-enriched oligoclonal bands (OCBs), in contrast to (4 %) (2/50) unrelated healthy controls. Furthermore, in this group of nine healthy sibs the measles CSF IgG antibody titers were higher than that of the other sibs and that of controls. There were also differences in the serum titers for measles IgG antibody, which were higher in the group of all healthy sibs than in healthy volunteers, and (as with CSF titers) higher in the subgroup of healthy sibs with two or more 2 CSF-enriched OCBs than the other sibs. Thus a significant proportion of healthy siblings to MS patients have a partially hyperimmune condition similar to that occurring in MS, which in 19 % manifested itself as an OCB reaction, in 9 % as increased CSF measles IgG antibody titers, and in 21 % as increased serum measles IgG antibody titers, these phenomena tending to occur in the same individuals. This condition is characterized by CSF-enriched OCBs with undefined specificity, although some increased antiviral reactivity is found both in the serum and CSF. While it needs further characterization, a genetic trait interacting with common infections is suggested. The recurrence risk of this condition is approximately five times higher than the 3–4 % recurrence risk for manifest MS reported for sibs.

Key words Multiple sclerosis · Siblings · Genetics · Oligoclonal bands · Measles

Introduction

Elevated measles antibodies in serum and CSF in multiple sclerosis (MS) patients were first demonstrated by Adams and Imagawa [1]. Subsequent studies have also shown elevated titers of measles serum and CSF antibodies in healthy siblings of MS patients, but data from healthy unrelated controls were provided only for serum [6, 13, 24, 34]. Furthermore, an intrathecal immunological reaction with oligoclonal bands (OCBs) has been found in healthy dizygotic twins to MS patients [35]. Poser has proposed the term "MS trait" for this partially hyperimmune phenotype [25, 26]. To determine the frequency of this condition we examined the CSF-enriched OCBs with IgG immunoblot and the CSF and serum viral antibody titers in 47 pairs of MS patients and their healthy sibs, and in 50 unrelated healthy volunteers.

Materials and methods

Ascertainment of patients, sibs, and volunteers

The dimension of the study was planned to be 50 patients with clinically definite MS (CDMS), their 50 healthy siblings, and 50 healthy unrelated volunteers, since this would allow detection of the one-fourth of phenotype-expressing individuals expected in recessive heredity. The study was approved by the Medical Ethics Committee

at the University of Gothenburg, and informed consent was obtained after written information. The MS patients were recruited from the Gothenburg MS register [28] and from local MS societies, yielding a sample of approximately 400 accessible MS patients. A letter was sent explaining the study, and from 200 answers obtained we initially included 50 consecutive eligible pairs of Scandinavian descent. When a CDMS patient had more than one sibling consenting to participate, we included the sibling whose age was closest to the patient's age. Three pairs were excluded, one of half-siblings, one of monozygotic twins, and one with symptomatic neuroborreliosis revealed during the present study. The results were therefore based upon 47 patients with clinically definite MS and their 47 healthy siblings.

A request for healthy volunteers free from current symptomatic disease was made at the Sahlgrenska University Hospital Blood Bank. Hospital staff was not invited to participate. We obtained 76 answers, and from these we included fifty consecutive volunteers in the study. Except for one volunteer who was of southern European origin, all were of Scandinavian descent.

A complete history was taken and a neurological examination was performed and scored with the Regional Functional Scoring System (RFSS), utilizing Kurtzke's Functional Systems in both patients and sibs [19]. Healthy volunteers were examined with ophthalmoscopy and blood pressure measurement. CSF and blood samples were obtained from all groups as an outpatient procedure for analysis of virus IgG antibody titers, total IgG and albumin assays, isoelectric focusing (IEF) with immunoblot, and CSF cell counts. In the subgroup with at least two OCBs in the healthy sibling we also analyzed measles IgM antibodies in the blood.

Basic and demographic parameters

Sex and age were equally distributed between the 47 patients and sibs, while the 50 healthy volunteers were younger (the possible effect of age was examined in subsequent analyses). Sex was unequally distributed between the sib pairs and the unrelated volunteers; the proportion of men was higher among the healthy controls (Table 1). Of the 47 patients with CDMS 3 were primary progressive while 22 were in the relapsing-remitting and 22 in the secondary progressive phase. Of the 47 sibs three suffered from migraine, three had had a hysterectomy, two were on antidepressive treatment, two were on antihypertensive treatment, and six had each one of the following: previous transient cerebrovascular disease, hip operation, ileus, orally treated diabetes, and well-controlled hypothyroidism. There was no age difference between the sibling group with fewer than two CSF-enriched OCBs (mean 45.4 years) and those with two or more (mean 45.7 years). In the group of healthy volunteers two persons were on antidepressive treatment, one had been operated on for lumbar disc hernia 1988, and one had a previous syncopal episode.

Table 1 Sex distribution and median age (range) in the analyzable material, 47 sibling pairs, and 50 unrelated healthy volunteers

	CDMS patients	Healthy siblings	Unrelated healthy volunteers
Women	31	29	15
Men	16	18	35
Total	47	47	50
Median age (range; years)	45 (21–64)	45 (18–66)	33 (18–57)

CSF

Quantitative determinations of albumin and IgG in serum and CSF were performed using the Behring nephelometer analyzer (Behringwerke). The CSF/serum albumin ratio [30] was calculated as: CSF albumin (mg/l)/serum albumin ×1000, and was used as a measure of blood-brain barrier (BBB) function. IEF of samples was performed in polyacrylamide gel, and the gels were silver-stained as previously described [33]. All samples were also transblotted onto Immobilon-P membranes (Mollipore) after IEF and immunoreacted using an antiserum against the gamma chain of human IgG (goat antihuman IgG; BioRad). One of the authors (L. R.), who was blinded to the clinical data, was responsible for the analytical procedures and the interpretation of the IEFs and immunoblots. The presence of two or more CSF-enriched OCBs on the IEF that positively were identified as IgG bands by the immunoblots was considered a sign of local IgG synthesis, with the precondition that there were no corresponding OCBs in the blood. Using the cutoff level of two or more 2 positively identified CSF-enriched oligoclonal IgG bands, the frequency of positive and negative IEF tests were determined in the MS and control study populations.

Viral serology

Serum and CSF samples from patients, sibs, and controls were analyzed simultaneously by enzyme-linked immunosorbent assay (ELISA) for IgG measles antibodies, together with positive and negative control sera. Antigen was prepared as described previously [12]. Briefly, cell cultures of human fibroblasts infected with strain Edmonston E139 were allowed to develop until a complete cytopathogenic effect was observed. Cells were scraped down, freeze-thawed, ultrasonicated, and centrifuged, and the supernatant was saved as antigen, which was passively coated on to microtiter plates (Nunc Immuno Maxisorp, Nunc, Gothenburg). Patient samples were incubated in twofold dilutions starting from 1/10 for CSF or 1/100 for serum. For determination of IgG antibodies, peroxidase-conjugated affinity-purified goat anti-human IgG antibodies (Jackson Immunoresearch, Gothenburg) were used, and the color reaction after addition of *o*-phenylenediamine and H_2O_2 was determined by a spectrophotometer (Molecular Devices VMax kinetic microplate reader). One of the authors (T. B.), who was blinded to the clinical data, was responsible for the analytical procedures and the interpretation of the ELISA. Titers were given as the highest dilution giving positive reaction as compared to the negative controls. The individual cutoff value for pathological titers of the CSF and serum measles antibody, corresponding to the 95 % percentile of the titers of the unrelated healthy controls, was a CSF titer higher than 20 and a serum titer higher than 6400.

IgM antibodies to measles were determined by immunofluorescence, using measles-infected GMK-AH1 cells as antigen. Serum samples were incubated in twofold dilutions, and fluorescein-labeled goat anti-human IgM liquid globulin (bioMeriux) was used as a conjugate.

Herpes simplex virus (HSV) serology was performed by the use of a HSV-type common antigen as described previously [4]. In brief, a DOC-solubilized membrane antigen of HSV–1-infected cells were used in ELISA, and the procedure was carried out as described above for measles serology as regards sample dilution, conjugate, substrate, and absorbance determinations. HSV–2 specific antibodies were determined using purified glycoprotein G2 as antigen [4]. IgG antibodies to varicella zoster virus were determined by ELISA, and to Epstein-Barr virus by immunofluorescence, using in-house routine tests. Antibodies to human immunodeficiency virus (HIV) and rubella were determined by commercially available IMX system assays (Abbot Diagnostics).

Statistical methods

Differences in proportions of persons with two or more OCBs between patients, siblings, and healthy volunteers were tested by Fisher's exact test ("CSF-enriched OCBs without unequivocal specificity in the CSF"). Measles and HSV–1 CSF and serum titers and antibody index were compared using the Mann-Whitney test and the Wilcoxon signed rank test, with stratification for sex ("Measles antibody titers in the CSF and serum," and "Relationship between the occurrence of at least two CSF-enriched oligoclonal IgG bands and measles antibody titers and indices"). The effect of sex was examined by the Mann-Whitney test. The effect of age was examined by regression analysis for the measles antibody variables, and by a *t* test for the presence of at least two 2 CSF-enriched OCBs. Comparison of RFSS scores in the patients whose sibs had at least two 2 CSF-enriched OCBs with patients whose sibs had not was performed with the Mann-Whitney test ("Quantitative neurological score").

Results

Borrelia, syphilis, and HIV antibodies in the serum and CSF

Serum and CSF specimens were negative for *Borrelia* antibodies, apart from four individuals. One sib had a history of neuroborreliosis with headache and slight cognitive symptoms which subsided after treatment, remaining low *Borrelia* antibody titers in serum and negative *Borrelia* titers in the CSF, and at least two CSF-enriched OCBs. This sib pair was excluded from the study. One MS patient (who had at least two CSF-enriched OCBs) and one healthy volunteer (with fewer than two OCBs) had slightly elevated *Borrelia* IgG titers in serum, and one sib (who had at least two OCBs) had slightly elevated IgG and IgM titers in serum. The anamneses of these three individuals revealed no previous clinical episodes suggestive of neuroborreliosis. All CSF samples from patients, sibs, and healthy volunteers were negative for *Borrelia* antibodies. Syphilis and HIV serology was negative in the CSF and serum in the three groups.

CSF-enriched OCBs without unequivocal specificity in the CSF

The presence of at least two CSF-enriched OCBs was observed in 45 of 47 (96 %) of CDMS patients, 9 of 47 (19 %, 95 % confidence intervals 8–30 %) of their sibs, and 2 of 50 (4 %) healthy unrelated volunteers. There was a significantly higher proportion of sibs with at least two CSF-enriched OCBs than of healthy volunteers (P=0.0196). Generally the healthy sibs with at least two CSF-enriched OCBs had fewer bands than the patients.

Measles antibody titers in the CSF and serum

In the subsequent sections, individuals with BBB lesion as defined by a CSF/serum albumin ratio above 9.8 were excluded from all results presented as measles CSF titers. In comparisons between patients and sibs the pair was excluded if either patient or sib had a BBB lesion [5].

Serum measles IgG antibody titers tended to be higher in the patients than in their sibs (P=0.096). There were significantly higher serum measles IgG antibody titers in sibs than in controls (P=0.017). However, the significance was entirely due to the difference among the men. The CSF measles titers were significantly higher in the patients than in their sibs (n=40 pairs, after exclusion of individuals with BBB lesion, $P< 0.001$), and tended to be higher in the sibs (n=42) than in the healthy volunteers (n=47, P=0.094). Serum IgM measles antibody titers, when analyzed in nine pairs in whom the healthy sib had at least two CSF-enriched OCBs, were all negative. Previous studies [6, 13] reported higher measles titers in women. This was confirmed in the present study for healthy unrelated volunteers only (P=0.05), while there was no significant sex difference of measles titers in the sibs or the patients. There was no significant effect of age on CSF or serum measles antibody titers.

A viral antibody index [11] was also used, but a substantial number of individuals with a CSF titer below 10 (not detectable) then had to be excluded because these individuals probably have very different true antibody ratios. The index provided the same information as the CSF antibody titers in this study.

Relationship between the occurrence of at least two CSF-enriched oligoclonal IgG bands and measles antibody titers

The relationship between the occurrence of at least two CSF-enriched OCBs and the measles antibody titers in the serum and CSF measles titer in the healthy siblings is displayed in Fig. 1. Measles titers were higher in the sibs with at least two CSF-enriched OCBs than in the sib group with fewer than two OCBs. The difference was significant both in serum (P=0.040, Fig. 2) and in the CSF (after exclusion of individuals with BBB lesion, n=9 and 33 in the two groups, P=0.0067; Fig. 2). The measles antibody titers were also higher in the siblings with at least two CSF-enriched OCBs than in the healthy unrelated volunteers (for serum samples, n=9 sibs and 50 volunteers; for CSF samples n=9 sibs and 47 volunteers, P=0.0039 for serum and P=0.0092 for CSF). Four healthy siblings (9 %) had pathological measles IgG antibodies titers in the CSF in the absence of a BBB lesion, and two of these had fewer than two CSF-enriched OCBs (Fig. 1). If these two were nevertheless considered to have an intrathecal reaction similar to MS, the proportion of healthy siblings with this condition would be (9+2)/47, or 23 %.

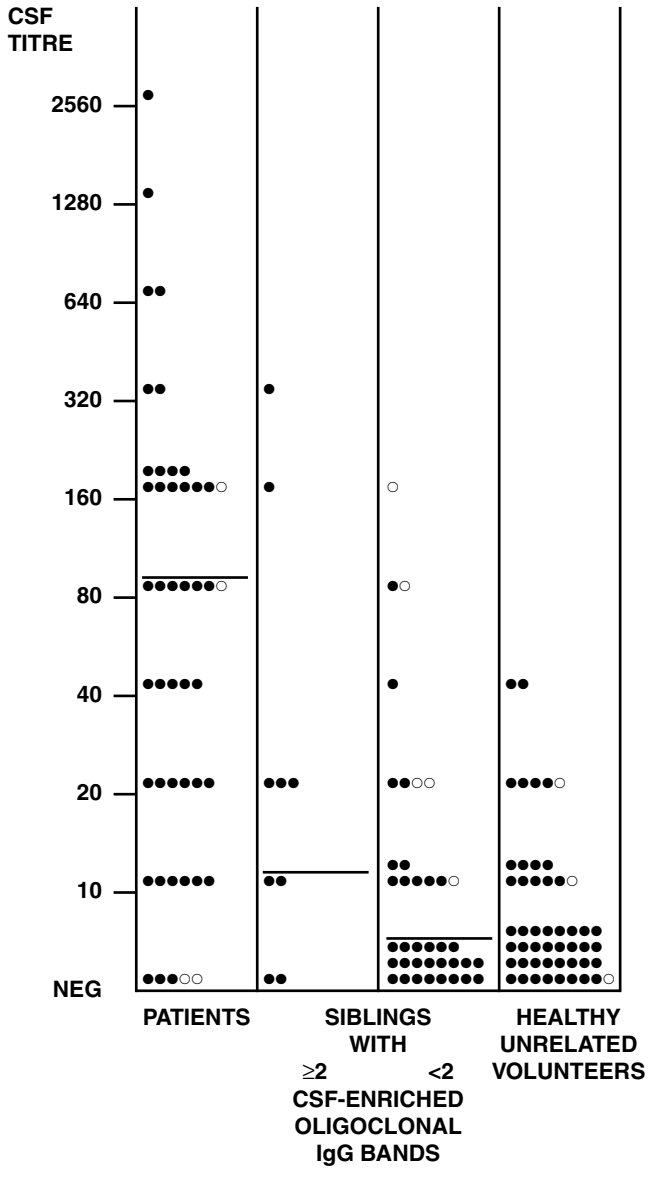

Fig. 1 Measles virus ELISA titers in CSF, in 47 CDMS patients, 9 siblings with at least two CSF-enriched OCBs, 38 siblings with fewer than two CSF-enriched OCBs, and 50 unrelated healthy volunteers. *Dots* Individuals; *circles* individuals with a BBB damage defined as an albumin ratio > 9.8; *horizontal bars* median values. The figure suggests a tendency to increasing titers through sibs with fewer than two CSF-enriched OCBs, sibs with at least two CSF-enriched OCBs, and particularly in CDMS patients

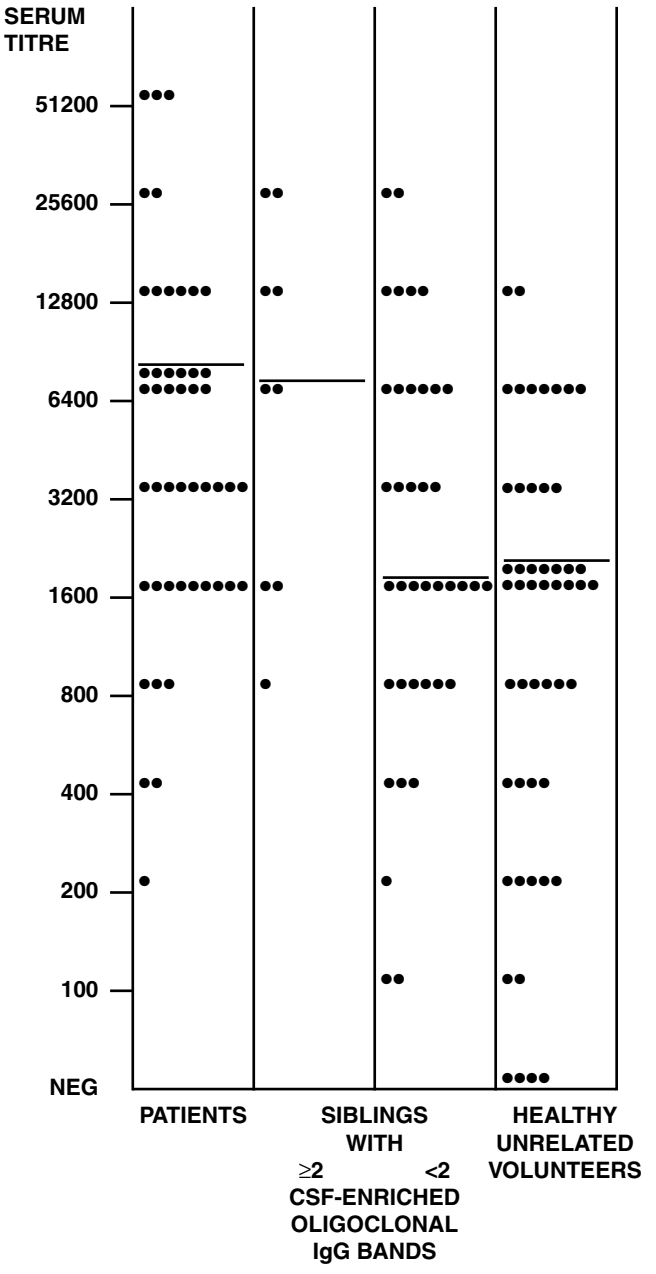

Fig. 2 Measles virus ELISA titers in serum, in 47 CDMS patients, 9 siblings with at least two CSF-enriched OCBs, 38 siblings with fewer than two CSF-enriched OCBs, and 50 unrelated healthy volunteers. *Dots* Individuals; *horizontal bars* median values. The figure suggests a tendency to increasing titers in sibs with at least two CSF-enriched OCBs and in CDMS patients

HSV–1 IgG antibody titer

The median CSF HSV–1 antibody titer in CDMS patients and healthy sibs after exclusion of cases with BBB lesion was 10, while it was negative for the healthy unrelated controls. The proportion of negatives was 19 of 43 (44 %) CDMS, 18 of 42 (43 %) healthy sibs, and 26 of 45 (58 %) controls; the range was negative to 160 in patients, and negative to 80 in both healthy siblings and controls. There were no significant group differences between the HSV–1 titers of healthy sibs and healthy unrelated volunteers in serum (P=0.52) or CSF (P=0.33). There was also no difference between healthy sibs with at least two CSF-enriched OCBs in this respect and the sibs with fewer than two CSF-enriched OCBs (P=0.52 for serum, P=0.38 for CSF) or from the healthy unrelated controls (P=0.49 for serum, P=0.32 for CSF).

Other viral antibody titers

Screening was also performed for HSV–2 CSF antibody in the group of nine healthy siblings with at least two CSF-enriched OCBs. These titers were all negative. The varicella zoster virus IgG titers were negative in six, and low in two individuals. The Epstein-Barr virus IgG titers were negative in three, and low in five individuals (ND=1). The rubella IgG titer was negative in 3 (ND=6).

Measles history and vaccination status

Measles was previously endemic in Sweden. A history of measles was confirmed in 40 of 43 CDMS patients, 38 of 45 healthy siblings, and 20 of 26 healthy controls who gave a precise statement on their measles history. Of 16 individuals who denied previous measles infection 9 had a history of measles vaccination. Measles vaccination was first introduced in Sweden in 1969 and was fully implemented by 1980 when all individuals were vaccinated both at age 2 and age 12 years. A precise statement on measles vaccination status was provided by 33 patients, 33 healthy sibs, and 24 healthy controls. Two patients, both with at least two CSF-enriched OCBs, 6 healthy sibs, and 6 healthy controls, all 12 with fewer than two CSF-enriched OCBs, had been vaccinated against measles. Thus the majority of healthy siblings with at least two CSF-enriched OCBs was measles unvaccinated.

Quantitative neurological score

CDMS patients having a sib with at least two CSF-enriched OCBs (n=9) and those without (n=38) had essentially the same median RFSS [18], of 11.77 and 10.53 (P=0.4).

Discussion

We found that 9 of 47 (19 %) of the healthy sibs to CDMS patients had an intrathecal immunological reaction with at least two CSF-enriched OCBs, distinct from 2 of 50 (4 %) unrelated healthy controls. Furthermore, in these 9 sibs the measles CSF IgG antibody titers were higher than in the other sibs and in controls. There were also differences in serum titers for measles IgG antibody, which were higher in the group of all healthy sibs than in healthy volunteers, and (as with the CSF titers) higher in the subgroup of healthy sibs with at least two CSF-enriched OCBs than the other sibs. Thus a significant proportion of healthy siblings to MS patients have a partially hyperimmune condition, in 19 % manifesting itself as an OCB reaction, in 9 % as increased CSF measles IgG antibody titers, in 21 % as increased serum measles IgG antibody titers. These phenomena tended to occur in the same individuals. Earlier studies were smaller, provided no serological data to exclude *Borrelia* and other CNS infections, and lacked normal CSF. In one study 5 of 28 (17 %) of healthy siblings of MS patients had abnormal CSF immunoglobulins [8]. We found a 4 % frequency of at least two CSF-enriched OCBs in our healthy volunteers. A frequency of 4–7 % intrathecal immunological reactions were found in healthy students and tension headache patients using IEF [16, 32]. However, specific serology was not reported in these studies either. Some asymptomatic persons with CSF-enriched OCBs were reported to develop later manifest MS [35]. One healthy sib who has been reported to have an oligoclonal CSF reaction [2] (not in the present series) now has CDMS. The proportion of sibs to MS patients presenting with CSF-enriched OCBs in this study (19 %) is much higher than the proportion of siblings to MS reported to develop manifest MS (3–4 %) [27]. With a median age of 44 years in the present study, our individuals carrying at least two CSF-enriched OCBs are by and large beyond the risk age of MS. Thus only a minority of this group could be presymptomatic MS.

Our patients tended to have detectable CSF IgG antibodies to HSV–1 more often than siblings and controls. However, only measles antibody titers were significantly increased in the present study. MS patients harbor immunological reactivity against a number of viral and myelin antigens, while viral, predominantly measles, antigens have been reported in healthy siblings [25]. Imprint electroimmunofixation may reveal the antibody specificity of individual IgG bands [22]. The virus-specific antibodies constitute only a minor fraction of the IgG. This method may be used to study the specificity in the group of healthy siblings with CSF-enriched OCBs.

This intrathecal reaction was apparently not the sequela of an intercurrent CNS infection (HIV, *Borrelia*, lues, or acute meningitis). We did not include individuals with a history or signs of previous CNS infections. Usually some specificity remains in the intrathecal reaction after neu-

roborreliosis, but sometimes only an unspecific oligoclonal reaction in the CSF remains [14, 15]. However, these infections are usually severe enough to be revealed by clinical history, leading to the exclusion of one sibling pair from the present study. A positive *Borrelia* serology in an MS patient with no suggestive features of the infection is unlikely to indicate neurological Lyme disease [7]. Notably, the healthy volunteer with low serum *Borrelia* titers in the present series was not one of the individuals with at least two CSF-enriched OCBs. One individual with low positive *Borrelia* titers in the serum was found in each of our three groups, approaching the number expected from the 1.3–1.4 % prevalence of serum *Borrelia* antibody-positive asymptomatic individuals in Gothenburg (Leif Dotevall, personal communication).

Could the MS-related partially hyperimmune state be identified by a noninvasive method, at least in the CNS compartment? Magnetic resonance imaging (MRI) detects subclinical abnormalities in the CNS. However, cerebrovascular disease is a differential problem in elderly persons. In previous studies two healthy siblings of 40 healthy relatives under the age of 50 years had MRI abnormalities, and 3 of 27 asymptomatic siblings had demyelinating MRI lesions, using strict criteria [20, 31]. In the British Isles survey only 3 of 33 discordant dizygotic twins showed MRI abnormalities fulfilling the Fazekas criteria [29].

The origin of this immunological reaction in MS patients and in their healthy sibs is unknown, but infectious and genetic factors and interaction between these two have been reviewed [3]. Twin studies [9, 21] suggest that both genetic and nongenetic factors are of importance. However, adoptee and half-sibling studies [10, 27] strongly indicate that the increased recurrence risk in siblings is predominantly genetic. We hypothesize that the analogously increased recurrence risk of CSF-enriched OCBs in siblings is genetically determined. Natural antibodies occur normally in low titers, which are genetically determined, and they may be important for control of early viral and bacterial distribution [23]. One way of differentiating antibodies in respect to their maturity concerning target recognition is analysis of the antibody avidity (which increases after the antibody recognized its antigen) using increasing salt concentrations in the antigen-antibody reaction [17]. It was reported that antibodies against measles in MS patients are predominantly of low affinity, while antibodies in patients with a primary viral infection are predominantly of high affinity [18]. Further characterization of the partially hyperimmune MS-related condition in larger materials should include viral and anti-myelin antibody specificity and avidity studies in the CSF and blood, cytokine patterns, and CSF markers for subclinical CNS damage, magnetic resonance spectroscopy, and HLA association studies.

In summary, we report that a MS-related partial hyperimmune state, which is predominantly but not totally confined to the CNS compartment, occurs in healthy siblings to MS patients with a considerable frequency, approximately five times higher than that of manifest MS in sibs. Pending further characterization of this condition, an analogy points to a genetic trait, or a genetic effect expressed in joint action with infections. However, data from the present study do not allow us to draw conclusions on the possible mode of inheritance of this condition. Both monogenetic inheritance with high gene frequency and polygenetic inheritance could explain our findings. Determining whether and how this phenotype is inherited requires family studies on its segregation. Further virological and genetic studies should compare the clinically asymptomatic and symptomatic subgroups of this MS-related hyperimmune state.

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