Common infectious agents in multiple sclerosis: a case–control study in children

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Environmental factors, in particular infections, have been linked with the risk of developing multiple sclerosis (MS). The association of Epstein–Barr virus infection with childhood onset of MS has been recently recognized. As other infections characteristically experienced during childhood have not yet been studied in larger cohorts of paediatric MS, we conducted a study on 152 German children with MS (age at onset <16 years) and matched controls in the hope of gaining evidence for their possible aetiological role in MS. Patterns of antibody responses were determined to a range of infections which, in prior studies principally on adult patients, had revealed possible associations with MS. In this study on children the serology of several infections showed associations with MS. In the exceptional case of *Chlamydia pneumoniae* there was a significantly higher prevalence of IgM antibody but, more typically, as in the case of influenza A, measles, parainfluenza 2, varicella/zoster viruses and particularly to the herpes simplex virus type 2 (HSV-2) lysate antigen, there were significantly higher concentrations of IgG antibody. Additional investigations, however, make it highly unlikely that a relevant number of children have experienced infections with HSV-2. In general this study supports and emphasizes a complex infectious and immunologic background of MS. *Multiple Sclerosis* 2008; 14: 136–139. <http://msj.sagepub.com>

Key words: childhood infections; Epstein–Barr virus; paediatric multiple sclerosis

Introduction

The epidemiological features of multiple sclerosis (MS) suggest that environmental factors influence the risk of the disease. In particular, the prevalence of MS is higher in those industrialized nations where infectious diseases are fewer and tend to occur later in life than in the developing nations. The serological evidence for past infections in MS patients as possible co-factors for MS has been investigated in many studies since the 1960s [1–7]. We investigated in children with MS and in sex- and age-matched controls concentrations of specific antibody against 10 common infectious agents, which had been described to be significantly elevated in MS patients by previous studies [3–7]. There

have been no reported associations with MS of any fresh or recent or reactivated infection (IgM antibody). Based on our own observations a possible exception might be *Chlamydia pneumoniae* (CP) and was therefore included in this study. Children with MS offer a unique opportunity to study a possible infectious background as most of the infections of putative relevance are experienced during childhood.

Methods

Groups of patients and controls, as described in a previous study [2], were increased in number to 152 in each group (Table 1). The mean age at the time

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of serum sample acquisition was 13.40 years for the MS patients and 13.45 years for the matched control subjects. Controls were recruited between 1989 and 2006 and none had suspected acute infections with the analysed agents.

Antibody detection and quantitation

Sera were obtained from the patients during their first stay in the hospital and were stored frozen below -20°C for subsequent analysis in parallel with the controls. All tests were validated for the intended use by the manufacturers as notified by the European Community (CE) sign. ELISA kits from Virion Serion®, Wuerzburg, Germany, were used to test for IgG antibody against adenovirus, herpes simplex 1/2, herpes simplex 2, influenza A, measles, mumps, parainfluenza type 2 and varicella; ELISA kits from Euroimmun, Luebeck, Germany, for IgG antibody against herpes simplex 1 gC1 and herpes simplex 2 gG2 recombinant proteins and an ELISA kits from Savyon Diagnostics distributed by Hain Lifescience, Nehren, Germany, were used to assay IgM and IgG antibody against CP. For the assays, 100 μL amounts of serum diluted 1:100 (or 1:500 for influenza A) were applied to antigen-coated wells. Colour was developed by the sequential addition of polyclonal anti-human IgG or IgM raised in

goats and *para*-nitrophenylphosphate according to the manufacturer's instructions. Software supplied by the manufacturer of the tests was used to calculate concentrations in Units/mL from the Optical density (OD) readings. IgG antibody to HHV-6 was determined quantitatively by ELISA, Panbio, Guilford Rd., Col USA, distributed by Viramed, Planegg, Germany. Rubella antibodies were assayed by a haemagglutination inhibition assay (in-house test) using human erythrocytes and preabsorption of the sera with kaolin. The level of the rubella antibody is expressed as a titre. Before testing for IgM all sera were preabsorbed to remove IgG. There were insufficient amounts of sera from 25 patients to test for adeno, influenza, parainfluenza, 24 for CP, 16 for HHV-6, 13 for mumps, 9 for rubella, 6 for measles and varicella and 3 for HSV-1/2 antibody, respectively.

Statistical analysis

Patients and controls were matched with respect to gender and age. Paired ranks-tests [8] were used to compare antibody concentrations, titre or standardized extinctions of ELISA values between the two groups. The Bonferroni-Holm procedure was used to adjust for multiple testing. For statistical analysis the SAS version 9.1 (SAS Institute Inc, Cary, NC, USA) was used.

Results

Concentrations of specific IgG antibody for influenza A, measles, parainfluenza 2 and varicella differed significantly between patients and controls. Patients also had higher levels of IgG anti-HSV-2 (lysate antigen) reactive antibody than controls (Table 2). Testing of those sera that were positive for IgG anti-HSV-1/2 ELISA (lysate antigen) for antibody against the individual recombinant viral proteins gC1 of HSV-1 and gG2 of HSV-2 revealed that all these children had experienced HSV-1 infections and that, additionally, five children (three patients and two controls) had antibody responses to the recombinant HSV-2 gG2 protein, though only weak. There was no evidence among either patients or controls for infection with HSV-2 only.

The great majority of the children with MS (95.6%) were positive for IgG anti-HHV-6 antibody. They did not differ significantly from the controls in respect to antibody prevalence and concentration (Table 2). The prevalence of detectable IgG antibody to CP was almost identical (49.2% and 48.0%, respectively). The IgM antibody prevalence

Table 1 Characteristics of paediatric MS patients ($n = 152$)

Characteristics	Number of patients	Percentage of patients
Gender		
Female	102	67.1
Male	50	32.9
Age at first attack (y)		
Mean (Median)	12.3 (13.1)	
Range	4.04–15.99	
Disease course at time of serum acquisition		
Relapsing remitting	149	98.0
Primary progressive	2	1.3
Secondary progressive	1	0.7
Serum acquisition: time from first attack (years)		
Mean (Median)	1.10 (0.43)	
Range	0.01–5.87	
CSF findings (available for 132 patients)		
Intrathecal IgG fraction >10%	95	72.0
Oligoclonal IgG	131	99.2
Immuno-modulatory or – suppressive therapy at the time of serum acquisition		
Corticosteroids	16	10.5
Beta-interferons	4	2.6
Azathioprine	2	1.3

Table 2 IgG antibody prevalences and antibody levels against common infections in comparison of paediatric MS patients with matched control subjects

Infection	Adenovirus*		HHV-6		HSV-1/2		HSV-2 ^f		Influenza A*	
	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
Number ^a	8	20	126	144	68	71	65	68	24	15
Prevalence (%)	6.3	13.2	95.6	94.7	51.0	46.7	43.5	36.2	18.9	9.9
Lowest level ^b	0.062	0.058	25	24	32	35	10	8	0.077	0.065
Highest level ^b	0.910	1.063	1310	1120	11410	4020	2883	435	1.115	1.390
Mean ^c	0.380	0.271	213	215	1410	951	448	119	0.294	0.298
Ratio of means ^d	1.402		0.990		1.483		3.765		0.987	
Median ^c	0.197	0.222	128	125	736	741	426	89	0.228	0.190
Ratio of medians ^d	0.887		1.024		0.993		4.787		1.200	
P-value ^e	0.40		0.20		1		<0.0001		0.035	
Infection	Measles		Mumps		Parainfluenza2*		Rubella		Varicella	
	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
Number ^a	70	137	74	122	27	30	101	123	88	147
Prevalence (%)	85.6	90.1	82.0	73.0	22.0	19.7	82.5	80.9	88.4	96.7
Lowest level ^b	181	180	74	77	0.074	0.105	8	8	103	106
Highest level ^b	9390	9910	5390	4800	1.087	0.788	512	256	6730	5490
Mean ^c	1469	1715	354	368	0.386	0.364	60	43	1283	655
Ratio of means ^d	0.857		0.962		1.011		1.395		1.959	
Median ^c	1165	1137	191	233	0.357	0.343	32	32	896	575
Ratio of medians ^d	1.025		0.820		1.041		1.000		1.558	
P-value ^e	<0.0001		0.29		0.029		0.054		<0.0001	

^anumbers of antibody positive patients and antibody positive controls.^bantibody levels.^cantibody concentrations of antibody positive, or of *standardized ΔOD-values of all subjects.^dratio of antibody levels of patients relative to controls.^eelevation of antibody levels in patients in relation to matched controls as analyzed in paired ranks-test, with application of the Bonferroni-Holm correction.^flysate antigen. Prevalences of IgG anti-HSV-2 gG2 antibody were 2.0 and 1.3%, respectively.

differed significantly between patients (28.9%) and controls (2.0%).

Discussion

The substantial evidence for an association of a previous EBV infection to MS was highlighted recently [9–11]. Two previous studies on paediatric MS patients have shown [1,2] that significantly more patients than controls had experienced remote EBV infection and, in the larger, more recently published study [2] it was concluded that possibly all paediatric MS patients had experienced remote EBV infection. Only two of the 147 MS patients were EBV antibody negative and these may represent the 'false negatives', which would be expected from the distribution of the antibody concentrations in a population of this size previously infected with EBV. Complex differences in antibody responses to some infections other than EBV have been reported between adult patients with MS and control subjects [3–7] and, in the present study, such differ-

ences have also been shown to occur in children. With the exceptions of EBV [1,2,9] and CP, the association with MS is only indicated by the slight though significantly elevated antibody concentration in the patients to certain infections: influenza A, measles, parainfluenza 2 and varicella. In contrast, we did not demonstrate significantly elevated titre or concentrations of IgG antibody to rubella, HHV-6 and adenoviruses in the patient group, possibly because of sample size limitation. Moreover, there are more than 60 types of adenoviruses, and association with MS may be restricted to just some types, which are not differentiated by the diagnostic antigen used.

Of all prior observations, those on herpes simplex serology in MS patients are the least clear [5,12,13]. The present study showed no association of HSV-1 with MS, whereas an MS-association seemed to be confirmed by the HSV-2 antibody test [3,12]. However, the HSV-2 lysate antigen ELISA is strongly cross-reacting with antibodies directed against the heterotypic virus in the patient as well as in the control group. The analysis of antibodies to the

recombinant gG2 proteins of HSV-2 revealed only for three patients and two control subjects evidence suggestive of a possible prior infection with HSV-2.

There have been many investigations on CP DNA in MS and a meta-analysis of inconsistent studies has recently been published [14]. An association of CP infection and MS is indicated in our study by a significantly higher prevalence of specific IgM antibody in the patients, an association that has not yet been reported in adult MS patients [15]. The high prevalence might be caused by a higher frequency for reactivation of this infection in the patients.

The data on different antibody levels indicative of infection by a range of pathogens in MS cannot be interpreted in a simple way that enables a deleterious or beneficial role to be attributed to a single agent. The finding that antibodies to only certain infectious agents are elevated suggests that different forms of repeated contact with the same or related antigen are a more likely explanation than polyclonal B-cell stimulation. Another possibility is a different timing of infections or vaccinations in MS patients and controls. Within the time period of this study children in Germany were mostly vaccinated against mumps, measles and rubella, while 10, 12 and 20%, respectively, still had histories of the natural infectious diseases [16]. In general, this study supports a complex infectious and/or immunological background of MS rather than the causative role of a single pathogen and, certainly, no single infectious agent has so far been shown to explain all aspects of the epidemiology of MS [6].

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