

Multiple sclerosis attacks are associated with picornavirus infections

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Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system, which often follows a relapsing–remitting (RR) course with discrete attacks. MS attacks have been associated with upper respiratory infections (URIs), but the specific viruses responsible have not been identified. We studied a cohort of 16 RRMS patients experiencing URI and followed them for clinically identifiable attacks. The viral causes of 21 separate URIs were investigated using culture and polymerase chain reaction (PCR) of nasal swab specimens, and by serology. Sibley's 'at-risk' period for MS attacks, beginning 2 weeks before and continuing for 5 weeks after a URI, was used for the analysis. Seven of the nine (78%) URIs due to picornaviruses were associated with an MS attack during the at-risk period. By contrast, only two of 12 (17%) picornavirus-negative URIs were associated with an MS attack ($P = 0.01$). The possible role of picornaviruses in the pathogenesis of MS deserves further study.

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Introduction

Multiple sclerosis (MS) is the most common neurological illness of young adult people, with a prevalence rate of approximately 50–100 per 100 000 persons.¹ Among the four clinical types of MS, relapsing–remitting (RR) disease is the most common.

Sibley *et al.* were the first to show an association between upper respiratory infections (URIs) and MS attacks.² This important finding was confirmed by subsequent studies, although no specific viruses were ever implicated.^{3,4} These studies defined 'at-risk' periods for MS attacks, extending 1–2 weeks before and 2–5 weeks after the onset of a URI. These at-risk periods encompass the incubation period (2–7 days), symptomatic period (1–2 weeks), and immune response to infections due to the common cold viruses, including rhinoviruses.⁵

There is a clear seasonal variation in the exacerbation rate of RRMS, with attacks occurring significantly more frequently in the spring and summer months.^{3,6,7} These findings were recently confirmed in a study showing a similar seasonal prevalence of enhancing MRI brain lesions in RRMS patients.⁸ Some viruses that cause URIs can also occasionally invade the central nervous system (CNS) and cause disease, including influenza A and B, coronaviruses, respiratory syncytial virus, and adenoviruses.⁹ The geographic distribution and apparent out-

breaks of MS are consistent with a viral trigger for this disease.¹⁰

We performed an observational cohort study of RRMS patients in an attempt to determine the viral causes of their URIs and to examine the possible association of particular viruses with MS attacks. A significant association between URIs due to picornaviruses and MS attacks during the at-risk period was observed.

Methods

Subjects and URIs

Patients with the established diagnosis of RRMS were eligible for the study if they presented with symptoms of a URI of 48 hours duration or less.^{11,12} URI was defined as the onset of or definite worsening of cold symptoms, including nasal congestion, nasal discharge, fever, cough, myalgias, and headache. That is, subjects were enrolled at or near the onset of URI and followed for the next 6–12 months. Each subject signed an informed consent document that had been previously reviewed and approved by the University of Utah Institutional Review Board. URIs were considered 'evaluable' if they reported the illness to the study staff, reported to the clinic, and had respiratory specimens taken. The subjects filled out a URI Symptoms Assessment form to collect information about the severity of their symptoms.

Follow-up for MS attacks

The timing and severity of MS attacks were determined by 1) direct questioning of the subjects during enrollment and subsequent (blood draw) visits, 2) reviews of the medical record, and 3) phone exit-interviews with the patients at the conclusion of the study (fall 2002). Attacks of MS were

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defined according to the criteria of Edwards *et al.*: 1) onset of new neurologic symptoms or 2) definite worsening of pre-existing symptoms lasting more than 24 hours and not related to fever.⁴ The data were analyzed using Sibley's definition for the 'at-risk' period for MS attacks, extending from 2 weeks before to 5 weeks after the URI.²

Virology and serology

Nasal swab specimens were collected in duplicate. One of the specimens was cultured for influenza A and B, RSV, adenovirus, and parainfluenza types 1–3 at the Utah State Health Department. The second specimen was frozen and sent to Respiratory Disease Study Unit, University of Virginia for the detection of picornaviruses (both human rhino- and enteroviruses) and human coronaviruses (strains 229E and OC43) by reverse transcriptase-polymerase chain reaction (RT-PCR).^{13,14} The primers and procedures for these PCR reactions have been previously described.¹⁵ Blood was collected from the subjects at 3-month intervals whenever possible. The serum specimens were tested for antibodies to seven respiratory pathogens (influenza A and B, RSV, adenovirus, and parainfluenza types 1–3) by complement fixation at ARUP Laboratories, Salt Lake City, UT. Serologic responses to picornaviruses and coronaviruses were not determined. The laboratories were blinded to information about the subjects, except for the date and source of the specimens. A serologic response to one of the viruses was defined as a fourfold or greater rise in titer between consecutive specimens.

Statistical analysis

The URI symptom severity scores were compared between groups using Student's *t*-test. MS attack data were organized into 2 × 2 tables and analyzed using the two-tailed Fishers exact test.

Results

Demographics

A total of 16 subjects ranging in age from 19 to 58 years were enrolled in this pilot study between October 2001 and April 2002. Thirteen of the subjects (81%) were female, similar to the general RRMS population. The study subjects were observed for an average of 8.8 ± 2.2 months (range 5.5–12 months). These subjects had had MS diagnosed for 7.3 ± 5.6 years and reported 1.3 ± 0.8 attacks per year. Twelve of the 16 subjects experienced only a single evaluable URI during the study, three subjects had two URIs, and one had three URIs.

Etiologies of the URIs

The 16 enrolled subjects experienced a total of 21 evaluable URIs during the study period. None of the nasal swab specimens examined by tissue culture were positive for influenza A and B, RSV, adenovirus, or parainfluenza types 1–3. Human coronaviruses were not detected by RT-PCR in any of the 21 specimens. However, human picornaviruses were detected by RT-PCR in nine of the 21 specimens (43%).

Thirty-six serum samples were obtained from the 16 subjects and examined for complement fixing antibodies to influenza A and B, RSV, adenovirus, and parainfluenza types 1–3. There was one seroconversion to RSV and three to parainfluenza viruses, all detected in blood taken in the spring of 2002.

MS attacks and URIs

Nine of the 16 (56%) subjects experienced a total of 19 MS attacks during 141 subject months of follow-up, yielding an overall attack rate of 1.6 per year (95% confidence interval 1.0–2.4/year). This is similar to the attack rates of about two per year found by previous investigators who used a similar definition for attacks.^{4,16} Nine of the 19 (42%) MS attacks occurred during the at-risk period, yielding a rate of nine attacks during the 34 subject months of follow-up within the at-risk period (0.26 attacks/month, 95% C.I. 0.13–0.44).² The remaining 10 MS attacks occurred outside the at-risk periods, which constituted the remaining 107 subject months of follow-up (0.09 attacks/month, 95% CI 0.05–0.17). Seven of the nine MS attacks that occurred during the at-risk periods were present at the time of enrollment and within 48 hours of the onset of URI symptoms. There was no correlation between the occurrence of MS attacks and the severity of the subjects' URI symptoms (data not shown).

Correlating specific viruses and MS attacks

There was a significant association between MS attacks during the at-risk period and the detection of picornaviral RNA in nasal swab specimens at the time of URI (Table 1). Seven of nine (78%) MS attacks were associated with picornavirus detection, while picornaviruses were detected in only two of 12 nasal specimens not associated with an MS attack ($P = 0.01$; RR = 4.7, 95% CI 1.3–17.5). Limiting the analysis to each of the 16 subjects' first URI only (excluding second and third URIs to reduce potential bias), five of seven picornavirus-positive URIs were associated with an MS attack during the at-risk period; none of the nine rhinovirus-negative URIs were associated with an MS attack ($P = 0.005$). Four picornavirus-associated MS attacks occurred in the fall/early winter and three occurred the following spring.

Effects of MS treatments on attacks and viral detection

Among the 16 subjects, nine were being treated with beta interferon and five were receiving glatiramer acetate at the time of enrollment. Two subjects were receiving no specific MS therapy. There was no apparent association between any of the MS treatments (individually or together) and the presence or absence of MS attacks during the study period. Similarly, there was no apparent

Table 1 Picornavirus isolation and MS attacks

	Picornavirus +	Picornavirus –	Totals
MS attack	7	2	9
No attack	2	10	12
Totals	9	12	21

association between MS treatments and the isolation of picornaviruses in these patients. However, this pilot study was not powered to detect differences between MS treated and untreated subjects.

Discussion

Picornaviruses, most likely all human rhinoviruses,¹⁴ were associated with MS attacks in this pilot study. Other URI pathogens, including coronaviruses, adenovirus, influenza viruses, RSV, and parainfluenza, were not detected in nasal wash specimens from the subjects with RRMS. This is the first study, to our knowledge, that directly implicates a single virus type in association with exacerbations of RRMS.

Several lines of evidence support the hypothesis that MS attacks may be caused by picornaviruses. First, the link between symptomatic URIs and MS attacks originally described by Sibley is well established, but the specific microbes, presumably viruses, responsible for this association have not been defined.^{2–4} Approximately 40%–50% of URIs are caused by rhinoviruses and 10–15% by coronaviruses.^{17,18} The ‘commonly’ cultured respiratory viruses (adenovirus, influenza A, influenza B, RSV, and parainfluenza viruses) account for less than 25% of human URIs and bacterial causes of URI are distinctly rare.¹⁹ The present trial applied reverse transcriptase-PCR for the detection of picornaviruses and coronaviruses, a technology that was not available to the previous studies linking URIs to MS attacks. It would be interesting to reanalyze respiratory specimens from these preceding studies looking for picornavirus RNA.

Secondly, MS attacks are clearly seasonal. An early description of the seasonality of MS attacks was provided by Sibley and Foley in 1965. MS attacks in their population had a bimodal distribution with peaks in the spring and again in the late summer and early fall.¹⁶ Subsequent studies have shown an increased incidence of MS attacks in the spring and summer months.^{6–8} The seasonality of MS attacks corresponds reasonably well to that of human rhinovirus infections, which also have an increased incidence in the spring and fall months.^{17,20} Other investigators have also commented on the similarities between the seasonal incidence of rhinoviral infections and MS attacks.²¹

Thirdly, a leucine-glutamate polymorphism at codon 469 in the human intercellular adhesion molecule-1 (ICAM-1) gene has recently been identified as a risk factor for the development of MS.^{22,23} Lysine/lysine homozygotes at this position in the ICAM-1 gene have a significantly increased risk of multiple sclerosis. ICAM-1 is the cellular receptor for approximately 90% of rhinovirus strains, suggesting that polymorphisms in this viral

receptor might account for part of the genetic susceptibility to MS through modulation of rhinovirus binding and infection.²⁴

The mechanisms by which picornavirus infections may cause MS attacks remain to be elucidated. These infections are known to provoke local inflammatory mediator responses (e.g., IL-1, IL-6, IL-8) in the upper respiratory tract that could trigger inflammation in the brain. However, we found a lack of any association between the severity of URI symptoms and MS attacks. That is, particularly severe URIs did not seem to predict MS attacks, although the power to detect such an effect was limited by the small size of the present study. Likewise, this study was not intended nor powered to detect an effect of the specific anti-MS drugs on the frequency of attacks.^a Alternatively, picornaviruses might transiently infect the brain itself, leading to an overly vigorous immune response within the parenchyma.

The apparent association between picornavirus-induced URI and MS attacks is a potentially important observation that might lead to advances in the treatment or prevention of MS attacks. The present study was limited by the relatively small sample size and the largely retrospective identification of MS attacks. These findings need to be confirmed by a second larger study.

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^aIt is worth noting that two of the three drugs approved by the US Food and Drug Administration for the prevention of MS attacks are interferon preparations. Interferons were originally described for their antiviral properties (i.e., they ‘interfere’ with cell-to-cell spread of the virus).²⁵

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