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Prospective study on the relationship between infections and multiple sclerosis exacerbations

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Summary

One of the characteristics of multiple sclerosis is the unpredictable occurrence of exacerbations and remissions. These fluctuations in disease activity are related to alterations in (auto-)immune activity. Exacerbations lead to short-term morbidity, but may also influence long-term disability. This longitudinal study in 73 patients with relapsing–remitting multiple sclerosis assessed the contribution of systemic infections to the natural course of exacerbations. In addition, we analysed whether infections lead to an increase in the number of gadolinium-enhancing lesions. A total of 167 infections and 145 exacerbations were observed during 6466 patient weeks. During a predefined at-risk period (ARP) of 2 weeks before until 5 weeks after the onset of a clinical infection (predominantly upper airway infections), there was an increased risk of exacerbations (rate ratio 2.1), which is in accordance with previous studies. Exacerbations with onset during the ARP led more frequently to sustained deficit [increase of ⩾1 Expanded Disability Status Scale (EDSS) point or ⩾0.5 above EDSS 5.5 for >3 months] than exacerbations with onset outside the ARP, with a rate ratio of 3.8. Minor and major exacerbations were equally distributed between the ARP and non-ARP onset groups. ARP exacerbations were associated with significantly higher plasma levels of the inflammatory marker soluble intracellular adhesion molecule 1 than non-ARP exacerbations, indicating relatively enhanced immune activation during ARP relapses. Three serial MRI scans were performed after the onset of an infection over a 6-week period. There was no difference in the number of gadolinium-enhancing lesions between the three time points. In conclusion, exacerbations in the context of a systemic infection lead to more sustained damage than other exacerbations. There is no indication that this effect occurs through enhanced opening of the blood–brain barrier.

Keywords: multiple sclerosis; disease course; infection; inflammation; MRI; sICAM; exacerbation

Abbreviations: ARP = at-risk period; BBB = blood–brain barrier; EDSS = Expanded Disability Status Scale; ELISA = enzyme-linked immunosorbent assay; ERV = exacerbation-related visit; IFN-β = interferon-β; IL-12 = interleukin 12; IRV = infection-related visit; MP = methylprednisolone; RR = relapsing–remitting; RV = regular visit; sICAM-1 = soluble intracellular adhesion molecule 1

Introduction

Multiple sclerosis starts in 80–85% of patients with a relapsing–remitting (RR) course (Weinshenker *et al*., 1989*b*; Noseworthy *et al*., 2000). Exacerbations can affect normal daily life substantially through the impact of a sudden, unexpected impairment. The unpredictable character of the onset and duration of impairment provokes uncertainty. Frequently, recovery is incomplete, resulting in sustained neurological deterioration (Lublin *et al*., 2000). Little is known about what initiates exacerbations and which natural processes determine the level of recovery, but immunological mechanisms seem to play a major role. There is good evidence that clinical manifestations of exacerbations are the result of focal areas of inflammation that block impulse conduction. This block is caused by direct effects of inflammatory mediators, the demyelination of axons or both (Smith and McDonald, 1999). The central role of the immune system in the pathogenesis of exacerbations is further supported by the observed influence of clinically manifest infections. These situations of increased release of inflammatory mediators increase the exacerbation rate (Sibley *et al*., 1985; Andersen *et al*., 1993; Panitch, 1994; Edwards *et al*., 1998).

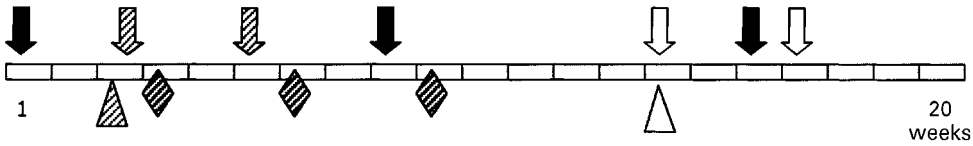

Fig. 1 Schematic representation of patients' visits during the study. Patients were seen at the out-patient clinic at regular intervals of 8 weeks for a regular visit (filled arrows). For every confirmed infection (hatched triangles), two additional infection-related visits were organized (IRV1 and IRV2) 3 weeks apart (hatched arrows). Likewise, for every exacerbation (open triangle) there were additional exacerbation-related visits (ERV1 and ERV2) (open arrows). Following infection, three MRIs were performed (hatched diamonds) over ~6 weeks.

In addition to the short-term impact of exacerbation activity, an even greater problem for multiple sclerosis patients is the long-term deterioration. Sustained clinical deterioration occurs after irreversible demyelination or loss of axons in a clinically eloquent neuroanatomical area (Trapp *et al*., 1999). It is still a challenge to find biological determinants of this sustained pathology. Genetic factors probably play a role (Dyment *et al*., 1997; Sawcer *et al*., 1997; Willer and Ebers, 2000; Chapman *et al*., 2001), but there may well be other, environmental factors at play. The relationship between exacerbations and long-term disease progression is incompletely understood. A high exacerbation rate during the early course is associated with worse prognosis (Weinshenker *et al*., 1989*a*), although this effect seems to be lost in the secondary progressive phase (Confavreux *et al*., 2000). This may suggest that, at least in the RR phase, the level of inflammatory activity influences the extent of structural brain damage (Bitsch *et al*., 2000). Thus, any factor that increases inflammatory activity could contribute to neurological deterioration. Systemic infection might be such a factor.

In this longitudinal study, we closely monitored 73 patients with RR multiple sclerosis in order to better understand the relationship between infection and exacerbation. We placed special emphasis on the question of whether infection-associated exacerbations lead to more severe and/or sustained neurological damage than exacerbations that are not associated with infections. To assess the influence of infections on the breakdown of the blood–brain barrier (BBB), we performed gadolinium (Gd)-enhanced MRI (Kermode *et al*., 1990; Miller *et al*., 1991). We determined plasma levels of soluble intracellular adhesion molecule 1 (sICAM-1) (Giovannoni *et al*., 1997, 2001; Rieckmann *et al*., 1997) and interleukin-12 (IL-12p40) (van Boxel-Dezaire *et al*., 1999) as surrogate markers of inflammatory activity. These markers have been shown previously to be associated with increased disease activity in multiple sclerosis.

Patients, study design and methods

Patients

Eligible subjects were patients aged between 18 and 55 years who had clinically definite or laboratory-supported multiple sclerosis (Poser *et al*., 1983) and an RR course (Lublin and Reingold, 1996) and had had at least two exacerbations in the previous 2 years. Patients who had other serious conditions concomitantly were excluded from the study. All patients gave written informed consent. The medical ethics committee of our hospital approved the study protocol.

Study design

The Rotterdam Study on Exacerbations (ROSE) was a longitudinal, prospective study that took place between July 1997 and December 1999. Patients were examined at our out-patient clinic during regular visits (RV) every 8 weeks (Fig. 1). They were instructed to contact the study centre by telephone as soon as they experienced symptoms of infection or neurological impairment. In case of a suspected infection or exacerbation, an additional visit to the out-patient clinic was arranged within 3 days. If the event fulfilled the criteria defined below, this visit was termed an 'infection-related visit 1' (IRV1) or an 'exacerbation-related visit 1' (ERV1). A second visit was arranged 3 weeks after the IRV1 or ERV1 and was termed the 'follow-up infection-related visit' (IRV2) or 'follow-up exacerbation-related visit' (ERV2).

Scores on Kurtzke's Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) and the functional system subscores were determined at every RV, ERV1 and ERV2. Blood samples were taken at every visit. To control for adequate reporting to the clinician, patients kept a weekly diary throughout the complete study period, in which they reported infections and neurological complaints. At the end of the study, there were no infections or exacerbations mentioned in the diaries that had not been reported to the clinician immediately after onset. In case of confirmed symptomatic infection, a sequence of three MRI scans was made, the first (MRI1) as soon as possible, the second (MRI2) 3 weeks and the third (MRI3) 6 weeks after the onset of infection. An MRI control group was set up, consisting of nine patients who underwent monthly MRI scans for a period of 8 months (55 MRI scans were performed; 17 time-points were missed).

Definitions

Exacerbation was defined as worsening of existing symptoms or the appearance of new symptoms lasting >24 h after a

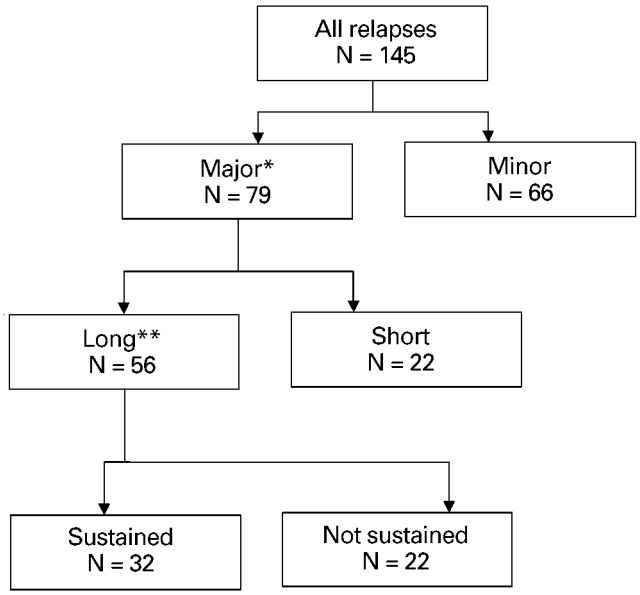

Fig. 2 Distribution of clinical subtypes of exacerbations. *For one major visit, no RRV2 data were available; **for two major long exacerbations, with onset towards the end of the study, there was insufficient follow-up (<3 months).

period of ⩾30 days of improvement or stability (Schumacher *et al*., 1965). Neurological deterioration only temporarily associated with a period of fever was not considered as exacerbation. Infection was defined as the appearance of coryza, sore throat, flu-like feeling, myalgia, fever, diarrhoea or a urinary infection lasting >24 h. Exacerbations were categorized according to severity into major and minor exacerbations. Major exacerbations had an increase of ⩾1 point on the EDSS (⩾0.5 point above EDSS 5.5) since the preceding visit. An attack of optic neuritis with an increase in the functional system subscore of ⩾3 points was also considered a major exacerbation. A minor exacerbation was defined as an exacerbation with an increase of ⩾1 point in any of the functional system subscores but not leading to a change in EDSS.

The major (but not the minor) exacerbations were also categorized according to duration into short and long exacerbations (Fig. 2). In short exacerbations, the EDSS recovered to pre-relapse level at the follow-up visit 3 weeks later (ERV2). In long exacerbations, the worsening in EDSS lasted beyond ERV2 (3 weeks). Next, the long relapses were subclassified as either sustained, with the worsening in EDSS remaining for ⩾3 months (IFNB Multiple Sclerosis Study Group, 1995), or not sustained, with recovery within 3 months after onset.

Methods

Cytokine measurements in plasma

Blood was collected into an 8 ml Vacutainer CPT sodium–heparin tube containing Ficoll (BD Biosciences, Erembodegem, Belgium). Plasma was aliquoted and stored at –80°C until use. Cytokine levels in plasma were determined by enzyme-linked immunosorbent assay (ELISA) for quantification of human IL-12p40 and a human sICAM-1 (Biosource International, Camarillo, Calif., USA), according to the manufacturer's instructions. Cytokine levels were calculated from the standard curves included in the assay kits.

MRI

A 1.5 T MRI system (Philips NT, Best, The Netherlands) was used to obtain SE T_1-weighted pre- and post-Gd-DTPA (diethylenetriaminepentaacetic acid) images [5 mm slices with 0.5 mm gap, TR (repetition time) = 450 ms, TE (echo time) = 15 ms, FOV (field of view) = 230 mm, matrix = 256 × 256]. In order to increase sensitivity, the MRI protocol was improved during the study: from February 1999, the dose of Gd-DTPA was raised from 0.1 mmol/kg (single dose) to 0.3 mmol/kg (triple dose) and T_1-weighted sequences with contiguous 3 mm slices (TR = 525 ms, TE = 13 ms, FOV = 230 mm, matrix = 256 × 256) were obtained. Single and triple-dose Gd series were analysed separately. Reproducible head positioning was achieved by the use of internal landmarks and comparison with the first MRI performed in a series.

Statistical methods

To assess the relationship between clinical infections and exacerbations, an 'at-risk period' (ARP) was defined as the period including 2 weeks before and 5 weeks after the onset of a clinical infection, as described previously (Sibley *et al*., 1985). For each patient, the ARP was determined and the number of exacerbations occurring within the ARP was counted, as well as the number of exacerbations occurring outside the ARP. Exacerbation rates during and outside ARPs were compared using Poisson regression analysis and exacerbation rate ratios were determined. This method was also used to study the occurrence of different subtypes of exacerbations.

To evaluate the relationship between the levels of immunological markers tested (sICAM-1 and IL-12p40) at various time-points during follow-up corresponding to the specific clinical status of the patient (i.e. remission, infection, exacerbation, ARP exacerbation), a repeated measures ANOVA, allowing for interindividual and intraindividual differences, was used (PROC MIXED procedure from SAS v. 6.12).

The MRI series were divided into two groups: those made after infections with no exacerbation and those made after infections with exacerbation in the ARP. The MRI parameters were tested with the χ^2, Wilcoxon and Mann–Whitney tests. The χ^2 test was used to test the difference in frequency of active MRIs between two groups, the Wilcoxon test was used for intragroup comparison between MRI1, MRI2 and MRI3, and the Mann–Whitney test was used for intergroup com-

parison between MRI1s, MRI2s and MRI3s. All analyses were performed using standard Stata Corporation (College Station TX, USA), SPSS Institute Inc. (Chicago, IL, USA) for Windows and SAS Institute Inc. (Cary, NC, USA) software.

Results

Patient characteristics

Seventy-eight patients were included (58 women and 20 men). Five patients withdrew after the intake visit. For the final analysis, follow-up data collected from 73 patients (56 female) were used. The average age was 39.9 years (range 19–55 years) and the average disease duration from diagnosis was 5.2 years (range 1–25 years). The average EDSS at entry was 2.6 (median 2.0, range 0–6.0) and the average number of exacerbations in the 2 years preceding enrolment was 2.2. Thirteen patients out of 73 (18%) withdrew before the end of the study. Of these 13 patients, 11 found the study visit schedule too demanding. One patient rapidly reached the secondary progressive phase and chose to participate in a bone marrow transplantation trial and one patient left the study for an unknown reason. A total of 124 patient years (6466 weeks) of follow-up were recorded, an average follow up of 1.7 years per patient. There was a total of 1234 visits to the out-patient clinic (approximately 17 visits per patient). Treatment with interferon-β (IFN-β) and methylprednisolone (MP) was allowed during the study. Of 73 participating patients, 15 (21%) were treated with IFN-β during the whole follow-up period and 14 patients (19%) changed the IFN-β treatment during follow-up, i.e. they started or stopped the treatment. In total, IFN-β was used during 28% of all follow-up weeks. Sixty per cent of the patients received no treatment with IFN-β. Pulse treatment with intravenous MP (3 days at 1000 mg per day) for exacerbations was given 55 times in 32 patients. No other disease-modifying drugs were used.

Infections and subclassification of exacerbations

A total of 167 infections was recorded in 86% of the patients, an average of 1.4 (range 0–4.5) infections per year. No significant difference in the yearly incidence rate for infections was found between patients with a low (EDSS <4.0) and a high (EDSS ⩾4.0) baseline level of impairment (an average of 1.3 and 1.5 infections per year, respectively). Of all infections, 77% were characterized by upper respiratory tract symptoms, 16% by gastrointestinal symptoms and 7% by urinary tract symptoms. In 25% of the infections, patients reported fever at some point during the course of illness.

A total of 145 exacerbations was recorded, occurring in 58 of the 73 patients and resulting in an average exacerbation rate of 1.2 per year (range 0–6.2 per year). Fifty-four per cent of all exacerbations were classified as major (79 of 145) and 46% as minor. Of all major exacerbations, 72% (56 of 78, follow-up for one of the 79 major exacerbations was not available) were long and 28% short. Thirty-two of the 54 major long exacerbations lasted >3 months and were classified as sustained, whereas 22 resolved within 3 months after onset (Fig. 2). For two of the 56 major long exacerbations, there was insufficient follow-up time for adequate classification, because they occurred towards the end of the study period. Forty-six out of the total number of 145 exacerbations (32%) started during the ARP. These exacerbations started on average 9.5 days after the clinical onset of infection, only 10 exacerbations (22%) preceding the onset of infection. Forty-five of all 167 observed infections (27%) were associated with an exacerbation.

Table 1 *Exacerbation rate during and outside the ARP*

Period	Number of weeks of follow-up	Number of exacerbations	Annual exacerbation rate
During at-risk period	1169	46	2.05*
Outside the at-risk period	5297	99	0.97

*Rate ratio 2.1 (95% CI 1.4–3.0, $P < 0.001$).

Increased risk of exacerbation around the time of infection

There was an increased risk of exacerbations around the time of infection (Table 1). This was the case for both major and minor exacerbations (exacerbation rate ratio 2.1 for both). When a narrower time window around infection was considered, as suggested previously (Edwards *et al*., 1998) (14 days before and 14 days after infection), the risk increased slightly to 2.4 [95% CI (confidence interval) 1.5–3.5, $P < 0.001$]. Specific focus on the weeks following infection showed a rate ratio of 2.2 (95% CI 1.4–3.3, $P < 0.001$) for the interval from week 1 to week 4 and 1.5 (95% CI 1.0–2.2, $P < 0.03$) for week 1 to week 8. To examine a possible delayed influence of infections on exacerbations, we also evaluated whether the period from week 3 to week 5 after infection was associated with an increased risk of exacerbation. The exacerbation rate ratio for this period was lower (1.6, 95% CI 0.9–2.8) and did not reach significance ($P = 0.09$). No specific type of clinical infection was associated with exacerbation. The distribution of clinical symptoms of upper respiratory tract infection, diarrhoea, flu and fever was equal between exacerbation-associated and non-associated infections. None of the urinary tract infections was related to an exacerbation.

Infection-related exacerbations are associated with prolonged neurological deficit

We observed a significantly increased risk of major long exacerbations during the ARP. Of 56 major long exacerbations, 21 started during and 35 outside the ARP, with yearly exacerbation rates of 0.9 and 0.3, respectively, resulting in a

Table 2 *Exacerbation rate during and outside the ARP for different types of exacerbation*

Exacerbation type	Number of exacerbations		Annual exacerbation rate	
	ARP (1169 weeks)	Non-ARP (5297 weeks)	ARP	Non-ARP
Major long	21	35	0.9*	0.3
Major sustained	15	17	0.7**	0.2

*Rate ratio 2.7 (95% CI 1.5–4.8, $P < 0.001$); **rate ratio 3.8 (95% CI 1.8–7.9, $P < 0.001$).

2.7 times higher exacerbation rate in the ARP (Table 2). The yearly exacerbation rates for major short exacerbations during and outside the ARP were equal (0.2 exacerbations per year).

Exacerbations leading to sustained deterioration were even more significantly associated with infections. The rate ratio for sustained exacerbations with onset during the ARP versus exacerbations with onset outside the ARP was 3.8, whereas exacerbation rates for non-sustained exacerbations showed no difference between ARP and non-ARP (Table 2).

Season does not influence the incidence of ARP exacerbations

To assess whether ARP exacerbations have a specific seasonal preference, we calculated the monthly incidence rate for infections, exacerbations and ARP exacerbations. The monthly incidence of infections was (average ± standard deviation) 0.11 ± 0.026, with the lowest incidence in July compared with the rest of the year (rate ratio 0.4, 95% CI 0.2–1.0, $P = 0.03$). As expected in this geographical area, there were significantly fewer infections in the summer months (June, July and August) than in the autumn months (rate ratio 0.67, 95% CI 0.4–1.0, $P = 0.04$). The average monthly incidence of exacerbations was 0.1 ± 0.021 and showed a peak in September, which did not reach significance compared with the rest of the year (rate ratio 1.56, 95% CI 0.9–2.5, $P = 0.08$). ARP exacerbations occurred with a constant average rate of 0.03 ± 0.014 per month without any peak during the year.

IFN-β and MP treatment do not influence the occurrence and type of ARP exacerbations

The possibility existed that the disease-modifying drugs used by a significant proportion of the study participants could influence the results, i.e. that their use could change the relationship between infection and the occurrence of exacerbations or subtypes of exacerbations. Therefore, we analysed the relationship between infections and exacerbations with respect to the use of immunomodulating drugs.

The frequencies of infections, ARP and non-ARP exacerbations and exacerbation rate ratios were not different between the IFN-β treatment and non-treatment periods, which is in accordance with another study (Panitch, 1994).

Similarly, MP, a drug known to shorten the duration of exacerbations, could theoretically have changed the clinical features of the studied exacerbations by causing a skew towards short-lasting exacerbations. However, the 45 MP treatments during this study were equally distributed between ARP and non-ARP exacerbations. In addition, statistical analysis with MP-treated exacerbations excluded still showed the association between infections and major long and sustained exacerbations.

Gd enhancement does not change after infection

In 101 out of 167 cases, three sequential MRIs were performed, starting immediately after the patients reported a clinical infection. The average intervals between the IRV to the out-patient clinic and the MRI scanning session were 7, 24 and 47 days for MRI1, MRI2 and MRI3, respectively. During the ARP there were exacerbations for 31 of 101 infections screened by MRI (on average 2.4 days after MRI1, range –25 to +36 days).

The percentage of scans with one or more lesions in the single-dose Gd series did not differ significantly between the different time points (18, 16 and 26%, $n = 60$, for MRI1, MRI2 and MRI3, respectively]. As expected, the percentages were higher for the triple-dose Gd series (38, 34 and 34%, $n = 41$), again with no significant difference.

The average number of Gd-enhancing lesions per MRI was 0.4 in single-dose and 0.8 in triple-dose Gd series. There were no significant differences between the numbers of lesions at the three different time points (Fig. 3). Also, the number of enhancing lesions did not increase when the infection was associated with an exacerbation. Excluding the MRIs from patients treated with IFN-β from the calculation (total 31 out of 101, 17 in single- and 14 in triple-dose Gd series) increased the average number of lesions measured but did not influence the overall result.

In the control group, nine patients underwent a monthly triple-dose Gd MRI during a period of 8 months. The average number of Gd-enhancing lesions was somewhat higher than the number of lesions seen on MRI performed around the time of infection, but the difference was not significant (mean 1.4, SD 2.6, $P = 0.1$). During this observation period, there were eight infections (in seven patients). Again, no significant

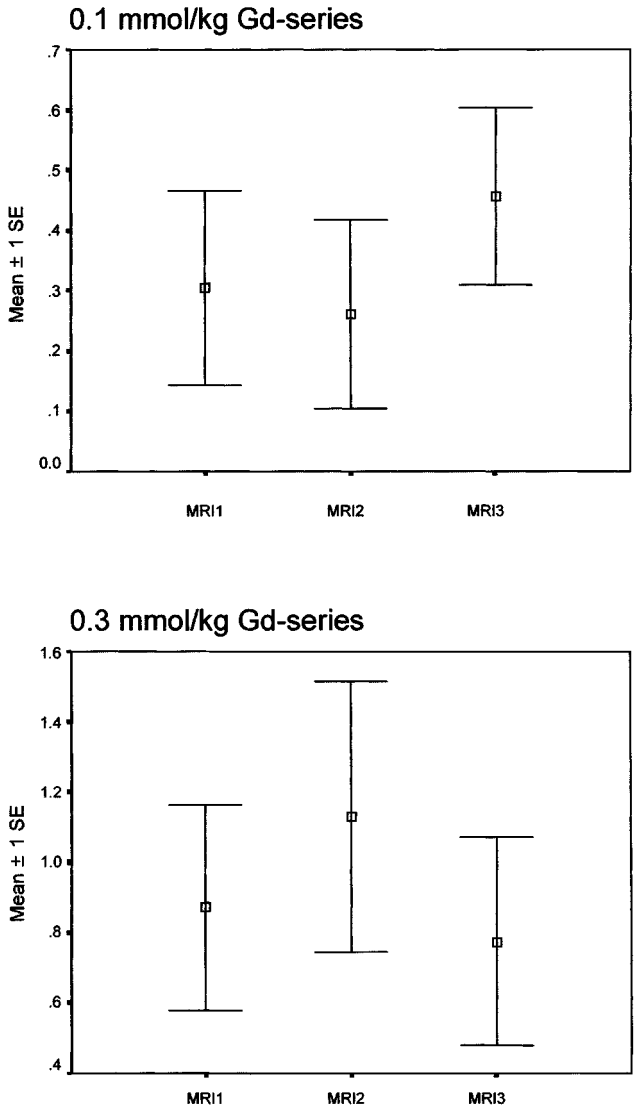

Fig. 3 Average number of Gd-enhancing lesions on three sequential MRIs after the onset of clinical infection. The number of Gd-enhancing lesions is shown on the *y*-axis. MRI1 was performed on average 7 days, MRI2 24 days and MRI3 47 days after the infection-related visit to the out-patient clinic.

change in the number of Gd-enhancing lesions was seen with respect to infections.

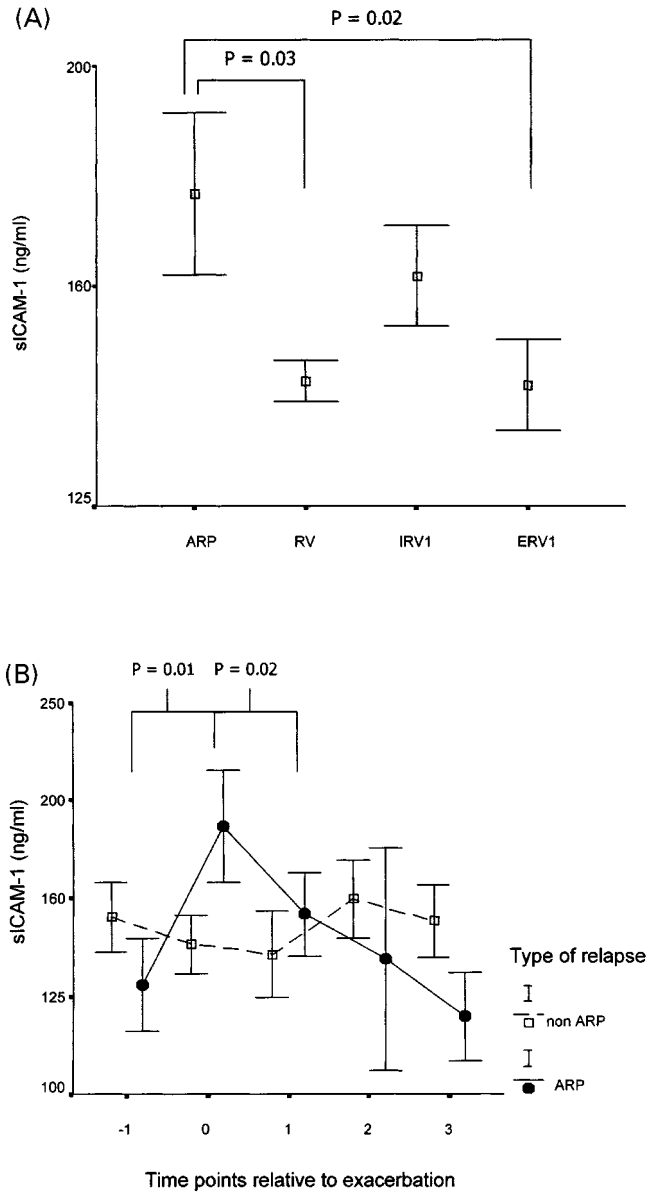

Fig. 4 Plasma concentrations of sICAM-1 (mean ± standard error). (**A**) At different time points during follow-up. ARP = ERV1 for exacerbations starting during the ARP (n = 26); RV = regular visit during disease remission (n = 319); IRV1 = infection-related visit 1 (n = 63); ERV1 = exacerbation-related visit 1 for exacerbations starting outside the ARP (n = 54). (**B**) Around the time of acute exacerbation. Time points: –1 = stable disease at regular visit preceding the exacerbation; 0 = exacerbation; 1 = visits 10–20 days after exacerbation; 2 = visits 21–30 days after exacerbation; 3 = visits >30 days after exacerbation.

Plasma levels of sICAM-1 and IL-12p40 in relation to exacerbations and infections

Plasma concentrations of sICAM-1 and IL-12p40 were measured at different time points during stable disease (RV), infection (IRV1), exacerbation (ERV1) or ARP exacerbation (ARP ERV1). Concentrations of sICAM-1 were significantly higher in samples collected during ARP exacerbations than during stable disease (P = 0.03) or non-ARP exacerbation (P = 0.02). However, levels of sICAM-1 were not different from levels observed during infections (IRV1) (Fig. 4A).

In addition, we performed a time-course analysis of sICAM-1 concentrations at the stages before, during and after exacerbations. In Fig. 4B, levels are plotted separately for ARP and non-ARP exacerbations. During ARP exacerbations, but not during non-ARP exacerbations, sICAM-1

concentrations increased significantly ($P = 0.01$ and $P = 0.02$ in comparison with preceding and following visits, respectively).

Plasma concentrations of IL-12p40 were found to be stable at all time points (data not shown).

Discussion

A main finding of this study is that exacerbations with onset around the time of a clinical infection lead to more sustained neurological damage than non-infection-associated exacerbations. Another important observation is the lack of support for the hypothesis that infections lead to an increase in Gd-enhancing lesions.

Apart from these observations, this study and previous reports on the relationship between infections and exacerbations (Sibley *et al*., 1985; Andersen *et al*., 1993; Panitch, 1994; Edwards *et al*., 1998) demonstrate a common pattern. Although the studies were performed in different geographical areas, there is a striking similarity in the observed enhanced risk of exacerbations during infections. The rate ratios were all within the narrow range of 1.3–3.4 (in this study 2.1). A common denominator of the different studies was that they were performed in temperate zones with a high incidence of multiple sclerosis (USA, Sweden, UK). In each study, it was concluded that the infections contributing to increased clinical disease activity were characteristically self-limiting, mild, non-specific upper airway infections and were supposedly of viral origin. In common with other studies (Andersen *et al*., 1993; Edwards *et al*., 1998), we found that the majority of infections preceded the neurological deterioration. Association with infection did not affect the severity of the exacerbations. This observation is not surprising, as one would predict the severity of an exacerbation to be dependent on the precise neuroanatomical location of the lesion rather than on the intensity or size of the inflammatory lesion.

So far, there has been no information available on the influence of clinical infections on the duration of exacerbations. Exacerbations can result from a variety of pathophysiological events, such as demyelination, inflammation, defects in synaptic transmission and the effect of circulating blocking factors. Recovery following exacerbation is a result of repair of axonal function through remyelination, the resolution of inflammation or the restoration of conduction to axons. Theoretically, the temporary release of circulating blocking factors during clinical infections might lead to short-lasting impairment associated with periods of increased conduction block (Smith and McDonald, 1999). Although most clinicians would call this a 'pseudobout', an impairment with duration >24 h would by definition still be counted as an exacerbation. Our study, however, shows that infections have more than a short-term effect. We demonstrated that infections were associated with a 2.7-fold increase in exacerbations that lasted >3 weeks (long exacerbations). More strikingly, sustained exacerbations with neurological deterioration lasting >3 months were 3.8 times more frequently associated with clinical infection. Although recovery can still occur after a 3-month period of sustained neurological deterioration, it has been suggested that this is rarely the case (Kurtzke *et al*., 1973). In fact, this is the reason why, in some clinical trials, the same definition—prolonged impairment for >3 months—was chosen as an outcome measure for treatment effects (IFNB Multiple Sclerosis Study Group, 1995). Thus, this sustained effect of infection-related exacerbations might well contribute to the long-term deterioration of multiple sclerosis patients.

Several studies have suggested that, especially in the more advanced phase of the disease, exacerbations do not influence further deterioration (Coles *et al*., 1999; Confavreux *et al*., 2000; Noseworthy *et al*., 2000). This is in contradiction with the experience of both clinicians and patients that phases of irreversible disease progression are often preceded by exacerbations. However, the exacerbations in the available natural history studies were not characterized intensively. In future studies it might be interesting to distinguish infection-associated from non-infection-associated exacerbations, to assess the relative contributions of each type of exacerbation to further permanent neurological damage in the progressive phase.

Current dogma considers BBB breakdown to be the initial pathological event of a clinical exacerbation (Kermode *et al*., 1990). The association of inflammatory activity with increased BBB breakdown, observed by Gd enhancement, is well established. As MRI is generally six to 10 times more sensitive than clinical assessment in detecting inflammatory disease activity (IFNB Multiple Sclerosis Study Group, 1995), we and others have hypothesized that, during infection, systemic immune activation increases Gd enhancement (Edwards *et al*., 1998). Our findings do not support this hypothesis, as there was no difference between the numbers of Gd-enhancing lesions immediately after infection and 7 weeks later. This is reminiscent of the lack of enhanced MRI activity after vaccination of multiple sclerosis patients (Michielsens *et al*., 1990; Salvetti *et al*., 1995). However, a major difference between the effects of vaccination and infection is that vaccination does not lead to increased clinical disease activity (Miller *et al*., 1997; De Keyser *et al*., 1998; Confavreux *et al*., 2001), whereas this study and others demonstrate significant positive effects on exacerbation rates. How can one account for the paradox that infections lead to increased lesional activity as reflected by clinical exacerbations but not as reflected by increased Gd enhancement? It has been demonstrated that pathological processes have already taken place long before lesions start to enhance (Filippi *et al*., 1998; Goodkin *et al*., 1998). This may imply that BBB breakthrough is a less crucial step in lesion pathology than was thought previously. Also, *in vivo* imaging of glial immunopathology with PET showed that during relapses there can be intensive lesional activity in areas remote from obvious inflammatory pathology (Banati *et al*., 2000). This is corroborated by recent pathological studies in which uncoup-

ling was observed between the inflammatory reaction associated with MRI enhancing lesions and ongoing CNS injury (Newman *et al*., 2001). It was suggested that this type of CNS damage is mediated by metalloproteinases that are active behind an intact BBB. Perhaps the phenomenon of CNS injury behind an intact BBB can explain the disappointingly low correlation between Gd enhancement and disease activity (Kappos *et al*., 1999; Koziol *et al*., 2001). Taking the available data together, we favour the interpretation that the contribution of infections to disease activity is related to components of an inflammatory process that occurs independently of BBB dysfunction.

According to the concept of an autoimmune pathogenesis for multiple sclerosis involving Th1 cells (Hintzen and Polman, 1997; Laman *et al*., 1998), active infection may lead to increased production of cytokines such as IFN-γ and IL-12. This would in turn lead to enhanced disease activity (van Boxel-Dezaire *et al*., 1999). However, we did not detect increased IL-12p40 during infection-related exacerbations. This could mean that other pathways are involved. It is also possible that plasma is not the most adequate compartment for detection of IL-12p40, as interesting clinical correlations with IL-12 fluctuations were more readily observed using an mRNA approach (van Boxel-Dezaire *et al*., 1999). There was, however, a significant increase in sICAM-1 levels during infection-related exacerbations as opposed to the stable levels that were observed during other exacerbations and during remission. This finding strengthens the notion that these two types of exacerbations have different biological characteristics. The relative increase in the sICAM-1 level during infection-related exacerbations may reflect a more intense inflammatory reaction, leading to increased axonal damage. Interestingly, Giovannoni *et al*. (2001) recently showed that sICAM-1 levels were higher in patients who progressed than in those who did not.

The nature of the infectious agents associated with increased disease activity has not been identified in any of the studies on this topic. Given the character of the upper airway infections that enhance disease activity in all the studies, it seems likely that a variety of viruses or other pathogens that can mimic viral upper respiratory infections such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* are involved. It will be a challenge to determine whether some are more involved than others.

In conclusion, this study shows that exacerbations associated with clinically manifest infections induce more prolonged structural damage than other exacerbations. However, it is not certain to what extent this damage is really irreversible. It will be interesting to investigate whether RR patients could benefit from the prevention or treatment of mild airway infections. Identification of the most frequent microbiological culprits will be essential in the design of such a therapeutic strategy.

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