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A prospective study of acute idiopathic neuropathy. II. Antecedent events

J B WINER,* R A C HUGHES,* M J ANDERSON,† D M JONES,‡ H KANGRO,§ R P F WATKINS‖

From the Departments of Neurology, United Medical and Dental Schools, Guy's Hospital, London; Medical Microbiology,† University College Hospital and Middlesex School of Medicine, Public Health Laboratory,‡ Withington Hospital, Manchester; Virology, St Bartholomew's Hospital,§ London, and Medical Microbiology,‖ St George's Hospital, London*

SUMMARY The incidence of antecedent events and serological evidence of preceding infection were studied in 100 patients with acute idiopathic neuropathy and age and sex matched control subjects in South-East England. Symptoms of respiratory infections occurred within one month before onset of neuropathic symptoms in 38% of patients and 12% of controls ($p < 0{\cdot}001$) and symptoms of gastrointestinal infections in 17% of patients and 3% of controls ($p < 0{\cdot}005$). Immunisations, insect bites and animal contact were equally common in the patient and control subjects. Eight per cent of patients had undergone an operation within the preceding 3 months. Six per cent of patients had co-existing "autoimmune" diseases. Serological evidence of recent infection was identified in 31% of patients. *Campylobacter jejuni* (14%) and cytomegalovirus (11%) were both significantly more frequently demonstrated in patients than controls. Serological evidence of recent infection with mycoplasma (1%), Epstein Barr virus (1–2%) and parvovirus B19 (4%) was also identified in the patients but not more frequently than in the controls. Possible explanations for the association of these agents with acute idiopathic neuropathy include possession of antigens shared with myelin and inhibition of suppressor mechanisms.

At least half of the reported cases of Guillain-Barre syndrome (GBS) have been preceded by infections or other antecedent events during the few weeks before the neuropathy.[1] A large number of events have been incriminated and in one study of 1100 case records there were 735 possible associations.[2] These include a variety of different infections, vaccinations, metabolic and neoplastic conditions. The only two case controlled studies suggested a higher incidence of respiratory infections[3] and gastroenteritis[4] among GBS patients than in appropriate controls. Serological tests have implicated cytomegalovirus (CMV) and Epstein Barr virus (EBV) more frequently than other viruses.

Virus specific IgM antibody against CMV was detected in 33 of 220 GBS patients and against EBV in eight of 100.[5] Another frequently reported association is mycoplasma; 5 of 100 patients in one study had evidence of recent infection.[6] Recent *Campylobacter jejuni* infection was identified serologically in as many as 21 of 56 patients in an uncontrolled study.[7] In that study evidence of infection with campylobacter was associated with more severe disease but mild cases of GBS associated with the same organism have also been reported.[8]

Our study compares the incidence of antecedent events in 100 patients who were thought on presentation to have GBS with age and sex matched control subjects. Serological tests were undertaken to identify recent infection with a number of possible aetiological agents.

Address for reprint requests: Dr J B Winer, Department of Neurology, St Mary's Hospital, Praed Street, London W2 1NY, UK.

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Materials and methods

Recording of antecedent events Patients with acute idiopathic neuropathy were recruited from a prospective study in South-East England during 1983 and 1984. Diagnostic criteria at the time of presentation fulfilled conventional stipulations for Guillain-Barré syndrome and are detailed in a preceding publication.[9] Three of these patients subsequently pursued a chronic relapsing or progressive course. There were 63 males and 37 females aged between 2 and 79 years.

It was not possible to obtain a history of antecedent events from one ventilated patient who died and had no relatives. Blood was not obtained from one control, a child on whom no venepuncture was being performed. The study patients were questioned by one investigator about infections, operations, transfusions, vaccinations, insect bites and animal contact in the 3 months prior to the development of neuropathic symptoms. An upper respiratory tract infection was defined as an episode of cough, cold or sore throat lasting more than 24 hours. Gastroenteritis was defined as an episode of diarrhoea with or without vomiting lasting at least 6 hours. An operation was considered to require either a general anaesthetic or 24 hours admission to hospital. Details of past illnesses were recorded.

Patients were asked whether they kept pets or had been in the same room as an animal in the last 3 months. Three grades of animal contact were recorded: no contact, contact but not continuous and pet ownership.

The same investigator asked hospital control subjects identical questions. A surgical patient was identified in the same hospital at the same time who matched the GBS patient for sex and was within 10 years of the same age. The control was to have lumbar or cervical disc disease but where this was not possible subjects undergoing procedures such as hernia repair or stripping of varicose veins were chosen. Patients with inflammatory conditions were excluded from the control group.

Statistical analysis A Chi square test incorporating Yates' correction for small numbers was used to test the significance of differences in incidence of antecedent events between patients and controls. Relative risks of developing GBS following different types of infection were calculated using the method of Miettinen.[10] Values for McNemar's test for matched pairs are quoted where appropriate.

Outcome criteria The outcome of the neuropathy was examined by applying a disability scale[11] at both 3 and 12 months after the initial interview. Outcome was considered poor if patients remained bedbound at 3 months or incapable of manual work at 12 months and the frequency of poor outcome among patients with identified preceding infections was tested using Chi square.

Serology Serum from venous blood from both patients and controls was tested for antibodies to CMV, campylobacter, EBV, mycoplasma and parvovirus. The first sample of blood was drawn from patients at varying intervals after onset of neurological symptoms (2–60 days) and the second sample 3 weeks later. Only a single sample was available from controls.

Cytomegalovirus The sera of patients and controls were tested for CMV specific IgG and IgM antibody with a solid phase radioimmunoassay as previously described.[12] CMV antigen was fixed to the wells of microtitre plates and incubated with dilutions of human serum. The plates were washed and ^{125}I-labelled anti-human IgM or IgG was added. After a further incubation the bound radioactivity was measured in a gamma spectrometer and a virus specific binding index (SBI) was calculated for each serum dilution. An SBI of >2 was considered positive for antibody. The results are expressed as the reciprocal of the maximum titre of serum that was positive. False positive reactivity in the IgM test due to rheumatoid factor was excluded by serum fractionation prior to testing.[12] The presence of IgM antibodies was taken to indicate recent CMV infection.

Mycoplasma pneumoniae Antibodies to *Mycoplasma pneumoniae* were measured with a complement fixation test in microtitre trays.[13] Commercially available *Mycoplasma pneumoniae* antigen (Colindale) was incubated overnight with dilutions of test sera and three minimum haemolytic doses of complement. Sensitised red cells were added and sera inhibiting haemolysis at dilutions greater than 1 in 16 were considered positive. A greater than four-fold rise in the antibody titre between paired sera or a titre of >256 in a single serum was taken to indicate recent infection.

Campylobacter jejuni Complement fixing antibodies to a sonicate of eight strains of campylobacter including *C Jejuni*, *C coli*, and *C lardis* were sought, using the Whitechapel technique[14] employing microtitre trays and 1·25 minimum haemolytic doses of complement.[15] A titre of 1 in 4 or greater was considered evidence of recent infection.

Parvovirus B19 Evidence of recent infection with parvovirus B19 was sought with an antibody capture radioimmunoassay.[16] Briefly test sera were incubated with a solid phase coated with anti-IgM or anti-IgG antibody. The solid phase was then washed and incubated with viral antigen, anti-parvovirus monoclonal antibody and finally an ^{125}I labelled antimouse antibody. The bound radioactivity was measured and compared with a standard curve obtained by diluting serum containing 100 arbitrary units of antibody in antibody negative serum and expressed in arbitrary units. Sera containing >3 units of specific IgM and >2 units of IgG were considered positive for the antibody.

Epstein-Barr virus IgM and IgG antibodies to virus specific capsid antigen (VCA) and complement fixing antibodies to Epstein Barr virus nuclear antigen (EBNA) were sought by immunofluorescence. Sera that were positive for IgM antibodies to capsid antigen and negative for anti-EBNA antibodies were absorbed with heat aggregated IgG and retested to exclude a false positive result from rheumatoid factor.

Results

Antecedent events

(1) Infections A history of respiratory symptoms one month before onset of the neuropathy was given by 38% of GBS patients and 12% of controls while gastrointestinal infection was reported by 17% of patients compared with 3% of controls (table 1). The peak incidence of antecedent symptoms of infection was between one and 2 weeks prior to the onset of neuropathy (fig).

(2) Immunisations The GBS patients received slightly but not significantly more immunisations in the 3 months preceding onset of neuropathy than the controls (table 2).

(3) Operations Eight patients gave a history of an operation within 3 months of the onset of neurological signs. Of these, two also had evidence of recent infection (CMV and *Campylobacter jejuni*). Controls could not be usefully questioned about recent operations since they were obtained from surgical wards and therefore almost all were awaiting or recovering from operations.

Table 1 *History of symptoms of infection in 99 patients and matched controls*

Type of infection	Time (weeks)	History of infection* Patients (%)	Controls (%)	Relative risk	90% confidence interval
Respiratory	4	38	12	4·1	2·2–8·9
infection	13	47	35	1·5	0·7–10·1
Gastrointestinal	4	17	3	7·5	2·1–18·2
infection	13	21	6	3·3	1·5–7·9
Both	4	55	15	4·8	2·4–9·9
	13	68	41	2·1	1·1–3·5

*The figures quoted for patients and controls represented the percentage of each group that could recall respiratory or gastrointestinal infection in either the preceding 4 or 13 weeks before the onset of their neurological symptoms.

Table 2 *GBS patients and controls with history of immunisation within 3 months. Figures in parenthesis are the interval in days from immunisation to onset of neuropathy*

Patient	Vaccine	Control	Vaccine
1	Influenza (32)	1	Tetanus (15)
2	Influenza (58)	2	Poliomyelitis (56)
3	Typhoid/Tetanus (23) Yellow fever (30) Poliomyelitis (30)	3	Tetanus (1)
		4	Influenza (61)
4	Yellow fever (10) Tetanus (10)	5	Tetanus (81)
5	Cholera (78)		
6	Typhoid/Tetanus (65) Poliomyelitis (65)		
Total vaccines 10		5	
Total vaccinated 6		5	

(4) Animal contact No significant difference existed between patients and controls in their degree of contact with cats or dogs in the 3 months preceding their neuropathy.

(5) History of recent insect bites A history of recent insect bites was not significantly different between GBS patients (14) and controls (11).

(6) Previous illness among the study patients A variety of previous illnesses was mentioned by individual GBS patients. Those of possible immunological or neurological importance are listed in table 3.

Serology

Serological evidence of campylobacter infection was

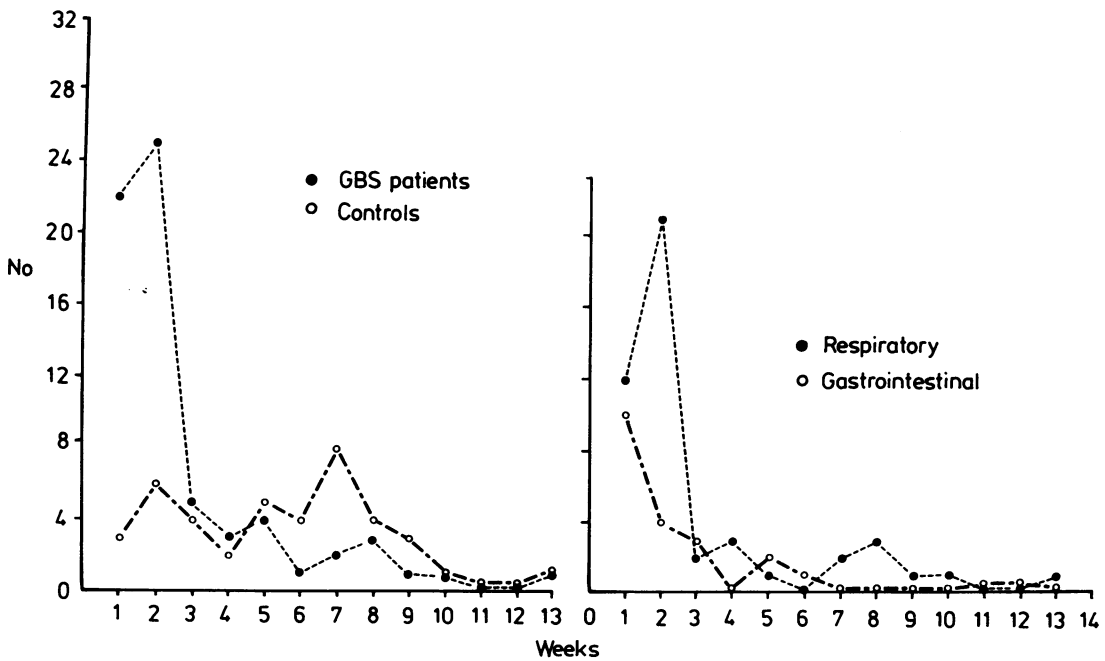

Fig *The number of patients or controls giving a history of antecedent infections at intervals before the onset of neuropathic symptoms is shown. The panel on the left compares patients with controls while that on the right gives the relative incidence of respiratory and gastrointestinal symptoms within the patient group alone.*

Table 3 *Associated illness of possible autoimmune or neurological importance*

Condition	*Number of study patients*
Psoriasis	4
Thyroid disease*	3
Idiopathic pernicious anaemia	2
Poliomyelitis	2
Rheumatic fever	2
Post-streptococcal glomerulonephritis	1
Myasthenia	1
Friedreich's ataxia	1

*Myxoedema 1, colloid goitre 1, benign thyroid nodule 1.

Table 4 *Percentage of positive serological tests*

	GBS patients (n = 99)	*Controls (n = 99)*	
Campylobacter jejuni	14	2	$p < 0.001$§
CMV IgG antibody	64	59	
IgM antibody	11	1	$p < 0.002$§
Parvovirus IgG antibody*	65	42	$p < 0.001$
IgM antibody*	4	0	
EBV definite†	1	0	
probable‡	1	2	
Mycoplasma pneumoniea	1	1	

*94 GBS and 67 controls tested (Chi square test)
†IgM and IgG antibodies to VCA, negative anti-EBNA
‡IgG antibodies to VCA, negative anti-EBNA
§McNemar's Test

obtained in 14 patients while IgM antibodies to CMV were seen in 11 patients. Both campylobacter ($p < 0.005$) and CMV ($p < 0.01$) infection were significantly more common among GBS patients than among controls. Recent infection with mycoplasma and EBV were each identified occasionally but not more often than in controls (table 4). Four patients but no controls had increased levels of IgM antibody to parvovirus B19 but this difference was not significant.

Outcome of patients with different antecedent infections
Only campylobacter and CMV infection were sufficiently common for meaningful analysis of differences in outcome. A higher proportion of those with serological evidence of campylobacter infection had a poor outcome at 12 months compared with the remainder ($p < 0.05$). Fourteen patients had serological evidence of recent infection with this agent. Of these three were dead at 12 months, six incapable of manual work and only five had made a good recovery. Recent cytomegalovirus infection did not significantly affect outcome.

Discussion

The incidence of respiratory infections among patients with GBS was first examined in a controlled prospective manner by Melnick and Flewitt.[3] Two control groups were questioned making a total of 71 subjects without central or peripheral demyelinating disease. One group of 48 was taken at random from patients admitted to medical wards and a second group of 23 in which respiratory diseases were excluded was also matched for age and sex. Antecedent respiratory infections within 3 months of onset of the neuropathy were reported by 50% of GBS patients compared with 32% of controls. A significant association was present for an interval of 1 month but not 3 months before onset of GBS. Our study confirms these findings on a larger patient population and a more rigorous control group matched not only for age and sex, but also for season and geographical location. In a retrospective study Kennedy *et al*[4] found an incidence of respiratory symptoms among GBS patients (38%) which was not significantly different from that seen in controls (27%). This may simply reflect the smaller number of patients or the fact that their data were less complete being obtained retrospectively.

None of Melnick and Flewitt's[3] patients had had preceding gastrointestinal illness. However Kennedy *et al*[4] reported gastrointestinal symptoms in 12 out of 37 GBS patients (32%) within one month of onset of neuropathic symptoms and in none of 37 controls with Bell's palsy. The controls were matched for age, sex and season. The GBS patients in our study reported a lower incidence of antecedent gastrointestinal symptoms (17%) within one month of onset of neuropathic symptoms which was nevertheless significantly higher than in controls.

Our results suggest that the greatest relative risk of developing GBS is seen in the first 2 weeks following infection. The median latency from infection to onset of neuropathic symptoms was shorter for gastrointestinal than for respiratory infections. Campylobacter was the major organism identified in association with gastrointestinal symptoms and was significantly associated with a worse outcome.

A report by Campbell[17] that six of 12 patients with GBS had had contact with feline enteritis prompted the investigation into animal contact. No association was detected. Similarly no significant association was detected between GBS and wasp or bee stings despite a report of an association of this antecedent event and GBS in some patients.[18] A few patients reported recent insect bites but not more commonly than controls. A radiculitis may follow tick bite induced borrelia infection in Bannwarth's lymphocytic meningoradiculitis or Lyme disease[19] but this was not recognised in our series.

The selection of appropriate controls for a study of this type is difficult because of the wide age range of patients and the possible role of infections in the aetiology of many comparable diseases. Kennedy *et*

al[4] used controls with Bell's palsy in their study, even though viral infections may have an aetiological role in Bell's palsy itself. Ideally several sets of controls would be obtained to reduce the chance that bias within a particular control group had unduly influenced the results. Our selection of surgical controls ensured a range of controls of appropriate age who were unlikely to have immunological disease. We intended to select patients who had been admitted to surgical wards with acute cervical or lumbar radicular pain of presumed mechanical origin. Unfortunately this objective was only achieved in about one quarter of the controls, while the remainder consisted of patients admitted to hospital for elective minor surgery. Surgical patients awaiting operation may report fewer antecedent infections since they represent a population screened for fitness for anaesthetic. Minor elective operations are likely to be postponed in patients with recent significant infection. Such screening generally applies only to the week prior to anaesthesia and it seems unlikely that this bias would account for our findings.

This study, in keeping with previous clinical experience,[1] identified a number of cases of GBS following surgery. Proof of this association being greater than chance would require further studies using a number of different control groups. Any association of GBS with operation is difficult to explain but may reflect an increased risk of infection during the post-operative period. Wound infection in one of our cases and blood transfusion in another provided possible routes of infection.

No association of GBS with vaccination could be demonstrated. Both GBS patients and controls reported equal exposure to immunisation although it is of interest that multiple immunisations were a feature of the history of three GBS patients. Kaslow *et al*[20] found no evidence that multiple doses of swine influenza vaccine were associated with a greater risk of disease than a single immunisation.

It is possible that the number of GBS patients surveyed was not sufficient to detect vaccine associated cases. The attributable risk of developing GBS following swine flu vaccination was estimated at 11·7 cases per million vaccinations[21] which would produce very few vaccine associated cases (assuming a low incidence of immunisation) in the 13 million population of our study area, even if our case ascertainment had been complete.

Our serological data indicate that campylobacter and CMV are the commonest infections identified in association with GBS, but these only account for a quarter of the patients. In the majority no preceding infection could be identified by the tests applied in this series. Occasional cases followed infection with EBV, mycoplasma and parvovirus B19.

A positive complement fixation test for campylobacter has been shown to correlate well with infection in the preceding 3 months.[15] A history of food poisoning was obtained in nine out of the 14 patients with serological evidence of recent *Campylobacter jejuni* infection and often other family members had been affected. This supports the reliability of our serological test for recent infection with this agent.

In the 11 patients with serological evidence of CMV infection, three reported respiratory, two reported gastrointestinal and one patient both respiratory and gastrointestinal prodromal illnesses. Virus specific IgM antibody in cases with no history of an antecedent illness is usually indicative of a primary infection but reactivation of latent CMV infection cannot be excluded.

An association of GBS with parvovirus B19 was investigated because a personal case of chronic demyelinating polyradiculoneuropathy followed documented parvovirus B19 infection presenting as Fifth disease (a mild febrile exanthematous disease seen usually in children and now recognised to be caused by parvovirus B19). Although IgM antibodies to parvovirus B19 were identified slightly more frequently in patients (4%) than controls (0%), the sera in these four cases were only weakly positive. It seems therefore unlikely that parvovirus B19 is a common antecedent infection. IgG antibodies to B19 were seen more frequently in GBS patients than controls but the significance of this finding is difficult to assess.

Our identification of only 1% to 2% of GBS patients with evidence of recent EBV infection contrasts with 8% of 100 patients in New Jersey discovered by Dowling and Cook.[5] This difference may reflect variations in age between patients in the two studies. Sixty two of our patients were over 40 years and only 11 under 20 years while primary EBV infection might be expected to be more frequent among children and young adults. The ages of the patients studied by Dowling and Cook[5] were not detailed and the criteria for diagnosis of infection with EBV were also different. All patients in our study were screened for antibody to EBNA. Recent primary infection was only diagnosed when IgM antibody to capsid antigen was present and antibody to EBNA absent. In addition sera that fulfilled these criteria were absorbed with heat aggregated to IgG to eliminate false positive results from IgM rheumatoid factor. Antibodies against EBNA were not sought by Dowling and Cook[5] who diagnosed infection on the basis of IgM antibody after absorption.

The lack of any resemblance between the two most common antecedent agents in GBS raises interesting questions concerning the mechanism by which infection triggers the disease. Homology between myelin

components and short sequences of proteins in both campylobacter and cytomegalovirus is one possibility by which T cell mediated damage of myelin might take place. Sequence homologies between both myelin basic protein and P2 protein and different virus proteins have been reported.[22 23] Alternatively an as yet unidentified infection might be common to all cases of GBS with other infections being coincidental. Another possibility is that infection triggers the neuropathy by interfering with suppressor cell circuits leading to a release of autoimmune phenomena. This may take place by specific mechanisms or a non specific adjuvant-like action. Such mechanisms are not mutually exclusive and the clinical syndrome of GBS may represent a common end point of a number of different pathogenetic mechanisms.

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