VIRAL INFECTION IN PATIENTS WITH MULTIPLE SCLEROSIS AND HLA-DR MATCHED CONTROLS

by D. A. S. COMPSTON, B. N. VAKARELIS, E. PAUL, W. I. McDONALD,
J. R. BATCHELOR and C. A. MIMS

(From the Institute of Neurology, Queen Square, London WC1N 3BG, the Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 0HS and the Department of Microbiology, Guy's Hospital Medical School, London Bridge SE1 9RT)

SUMMARY

Retrospective comparisons of the prevalence and age, where appropriate, of some childhood infectious illnesses and vaccinations, together with serological evidence for exposure to 16 viruses, many of which have previously been implicated in the aetiology of multiple sclerosis (MS) were made in 177 patients with acute optic neuritis, other recent isolated demyelinating episodes or established MS and 164 controls. The expected high frequency of HLA-DR2 in patients with demyelinating disease was matched by preselection of normal controls with this antigen (DR2+); the remaining individuals were classified as HLA-DR2 negative/DR3 positive (DR3+) or HLA-DR2 and 3 negative (DR2/3-). Cases were compared with controls, collectively and in analyses restricted to each genetic group; these comparisons were repeated considering the three categories of patients with demyelination and two control populations separately. All DR2+, DR3+ and DR2/3- individuals were compared in a single analysis to assess the effect of HLA type itself on the results.

Patients with demyelinating disease had rubella and measles at a later age and reported mumps infection more frequently than controls. Age of typhoid vaccination and duration of exposure to domestic dogs was higher in all cases than controls. Age of measles and mumps, but not rubella, was higher in DR2+ cases than controls; but differences were not observed in the other genetic groups. Higher rubella antibody titres were present in all cases than controls and in analyses confined to DR2+ individuals in whom higher Epstein Barr virus antibody titres were also present. Measles haemagglutination inhibition and parainfluenza I antibody titres were increased and influenza A antibodies detected less frequently in all patients with optic neuritis and those with DR2 compared with appropriate controls; influenza B antibody titres were lower in all DR2+ cases than controls. Higher adenovirus and varicella zoster antibody titres were present in DR2/3- patients with demyelination and other neurological diseases compared with normal controls. Overall, older age of infection and higher antibody titres were observed more often in patients with optic neuritis, in particular DR2+ cases, than other individuals with demyelination or controls.

Our serological results are consistent with the presence of abnormal HLA-immunological reactivity in patients with MS but cannot be explained only by an effect of DR type itself; age at which susceptible individuals develop some common childhood infections may also influence the subsequent development of the disease.

Correspondence to: Dr D. A. S. Compston, Department of Neurology, University Hospital of Wales, Heath Park, Cardiff CF4 4XW.

INTRODUCTION

Epidemiological evidence implicates a childhood infection in the aetiology of multiple sclerosis (MS). Age and genetically determined immune responsiveness could each influence the outcome of exposure to the putative agent, particularly if this is an ubiquitous infection, but direct evidence involving a specific organism either in initiating the disease or precipitating relapses is lacking.

The relationship between the HLA system and MS provides evidence for genetic susceptibility to the disease; in North Europeans the main association is with HLA-DR2 but there is also an increased frequency of HLA-DR3. This genetic heterogeneity may in turn reflect the aetiological role of different environmental agents, specific differences in the pathogenesis nevertheless leading to clinically indistinguishable diseases. It is probable that other genes interact with those encoded in the major histocompatibility complex in determining susceptibility to MS but at present the closest identified markers are the HLA-DR antigens themselves.

One approach to the investigation of environmental factors in MS has been to make viral and serological comparisons between patients with MS, cases with other neurological diseases and normal controls. But since genetic and environmental factors probably interact in the development of the disease, failure to compare MS patients with HLA matched controls may have obscured patterns of viral exposure which are only significant for the pathogenesis in individuals with the MS associated HLA-DR phenotypes. The existing studies cannot distinguish between clinical and serological abnormalities specific to MS and those which can merely be explained by an effect of HLA-DR type itself. In this study we have compared exposure to many organisms, some previously implicated in the pathogenesis of MS, in patients with optic neuritis, isolated demyelinating lesions, clinically definite MS or other neurological diseases and normal controls preselected to match the abnormal distribution of HLA-DR antigens found in individuals with demyelination.

METHODS

Patients and Controls

Idiopathic optic neuritis was defined as rapid onset of visual loss, usually with pain on ocular movement, and reduction in visual acuity or colour vision, excluding patients with other neurological symptoms or signs indicating previous or more widespread demyelination, or evidence for another condition which might cause optic nerve damage. The diagnosis of an isolated demyelinating lesion was made in patients with rapid onset of symptoms and signs referable to the brainstem or spinal cord, alone or in combination with cerebral and optic nerve disease, in whom investigations were consistent with demyelination. Patients with clinically definite (CD) MS were judged to be in relapse after developing one or more new, or a worsening of existing, symptoms lasting more than 24 h. These three groups are collectively referred to as patients with demyelination and all were aged less than 50 years.

Individuals with acute, stable or progressive diseases, excluding those known to be HLA associated,

caused by infection or the result of an immunological process were included as controls with other neurological diseases. This group included patients with migraine (20), structural lesions, including tumour (13), epilepsy (11), cranial nerve palsies (8), psychological or otherwise unexplained neurological symptoms (6), vascular disease (3), back pain (3), chronic renal failure (1) and muscular dystrophy (1).

Patients in each of these four categories were attending the National Hospitals for Nervous Diseases, Queen Square or Maida Vale, and Moorfields Eye Hospital.

Twenty-seven voluntary blood donation sessions in South London were attended during a ten-month preliminary period at which samples from 389 Caucasian individuals aged less than 50 years were typed for HLA-A, -B, -C and -DR alleles. Eighty out of 122 HLA-DR2 positive (DR2+) individuals agreed to participate in the study and a proportion of the 103 HLA-DR3 positive/DR2 negative (DR3+) and 164 HLA-DR2/3 negative (DR2/3-) individuals were also contacted to provide a control group of 37-50 individuals in each of these genetic groups.

Patients with demyelination were studied within six weeks from the estimated onset of new symptoms, together with 1 normal control; the group with other neurological diseases was not introduced until later in the study. Since the results of HLA typing in patients with acute demyelination were not immediately available, cases and controls studied at one time were not HLA matched. All participants were investigated during a consecutive 27-month period; 51 per cent were assessed between April and September and 49 per cent between October and March. Within each period the ratio of patients : controls remained constant.

A questionnaire was administered to each patient and control by one of us (B.N.V.) which assessed retrospectively the prevalence and age, where appropriate, of rubella, measles, mumps, varicella, herpes zoster, jaundice, conjunctivitis and any unidentified infectious illness in the three months prior to interview. Details of previous immunization against poliomyelitis, typhoid fever, tetanus, diphtheria, pertussis, measles, smallpox and tuberculosis were also obtained as was the estimated frequency and duration of contact with domestic animals; demographic and personal details were recorded. In some instances an estimate within various age limits was used to record age of infection. When necessary, relatives and general practitioners were contacted by the subject or interviewer to supplement the participants' replies, but this did not yield much additional information. An attempt was made to assess the reliability of answers by incorporating questions on topics for which there was no expectation of a difference between patients with demyelination and controls (e.g., the rate of skin cut healing).

A 30 ml sample of venous blood was taken for HLA typing and serological tests for antibodies against rubella, measles, mumps, varicella zoster, herpes simplex, influenza A, influenza B, parainfluenza I, II and III, adeno-, Epstein Barr, cytomegalo-, BK, OC43 and respiratory syncytial viruses.

A total of 341 individuals were studied; the 177 patients with demyelination included 64 with optic neuritis, 44 with isolated demyelinating lesions and 69 with CD MS in relapse. Of the 164 controls, 66 had other neurological diseases and 98 were normal blood donors. The number of DR2+, DR3+ and DR2/3- individuals in each clinical group is shown in Table 1.

TABLE 1. NUMBER OF INDIVIDUALS STUDIED IN EACH CLINICAL AND GENETIC GROUP

	<i>All cases</i>	<i>DR2+</i>	<i>DR3+</i>	<i>DR2/3-</i>
Optic neuritis	64	29 (45 %)	15 (24 %)	20 (31 %)
Isolated demyelinating lesions	44	23 (52 %)	6 (14 %)	15 (34 %)
Clinically definite MS in relapse	69	37 (53 %)	17 (25 %)	15 (22 %)
Other neurological diseases	66	20 (30 %)	16 (24 %)	30 (46 %)
Normal controls	98	57 (58 %)	21 (21 %)	20 (21 %)
Total	341	166 (49 %)	74 (22 %)	100 (29 %)

TABLE 2. METHODS FOR DETECTING VIRAL ANTIBODIES IN SERUM

<i>Test</i>	<i>Virus</i>	<i>Source of antigen</i>	<i>Specificity of test</i>
1. Complement fixation (CF)	Varicella zoster (VZ)	Flow Laboratories Ltd, Uxbridge, Middlesex	Specific (some cross reactions between these viruses)
	Herpes simplex (HSV)		Cross reactions between different adenoviruses
	Adenovirus		Cross reactions between parainfluenza viruses
	Parainfluenza 2 and 3		Specific for V antigen
2. Haemagglutination inhibition (HAI)	Mumps	Microbiological Associates, Maryland, USA	Specific
	Influenza A	Wellcome Laboratories, Beckenham, Kent	Specific
	Influenza B		Specific
	Measles	Behring Institute, West Germany, courtesy of Dr Gisela Enders	Specific
	Parainfluenza 1 BK	Sendai virus infected allantoic fluid Partially purified virus from human embryo lung cells (Courtesy of Dr D. McCance, Guy's Hospital Medical School)	Specific
3. Fluorescent antibody (FA) (indirect method* infected cells in PTFE-coated multispot slides obtained from C. A. Handley, Essex Ltd)	Epstein Barr (EB)	EB-infected 95/8 cells	Specific for viral coat antigen
	Cytomegalovirus (CMV)	CMV-infected MRC5 cells	Specific (anti-human C ₃ method)
	OC43	OC43-infected mouse embryo brain cells	Specific
4. Single radial haemolysis (SRH)	Respiratory syncytial (RS)	RS-infected Vero cells	Specific (bovine anti-RS serum*)
	Rubella	Rubiscreen plates (Northumbria Biologicals Ltd)	Specific

* Fluorescein isothiocyanate conjugated anti-human IgG, anti-human C₃, and anti-bovine IgG conjugates were obtained from Wellcome Laboratories, Beckenham, Kent

A higher proportion of females with demyelination was studied (2.12 F : M) than expected in patients with MS overall. This bias was not evident until completion of the study and was not corrected in the selection of controls (1.12 F : M); the excess of females was present in most groups of patients compared with either control population, considered collectively or in each genetic group. Mean age at the time of study was 32.2 ± 7.6 years in cases and 33.2 ± 8.8 years in controls; no age differences were present between the patients with optic neuritis (31.7 ± 8.2 years), isolated demyelinating lesions (31.2 ± 7.8 years), CD MS in relapse (33.3 ± 6.9 years), other neurological disease (32.4 ± 9.5 years) and normal controls (33.8 ± 8.3 years).

HLA Typing

HLA-DR antigen typing was done using the standardized Seventh Histocompatibility Workshop method with sera defining HLA-DR-7-7 and 9, which were donated by other laboratories or obtained locally. In each case, these antibodies were used in the Seventh or Eighth Histocompatibility Workshops or evaluated against Workshop reagents.

Viral Serology

All tests were carried out by standard methods as shown in Table 2.

Statistics

The results were analysed using the SPSS programme on the University of London CDC 6600 computer. The χ^2 test was used to analyse 2×2 or 2×5 contingency tables. The Mann-Whitney U test or Kruskal Wallis one-way analysis of variance was used to analyse the continuous variables which were not normally distributed. Most of the results presented in the tables relate to these continuous variables, such as age of infection or antibody titres. Since the methods used to analyse the results are not easily illustrated we have tabulated these data as relative risks for developing demyelination in three arbitrarily defined categories attempting to provide approximately equal numbers in each; individuals in whom antibody was not detected or there was no history of infection

TABLE 3. RELATIVE RISKS FOR DEVELOPING DEMYELINATION
AGE OF RUBELLA INFECTION

	<i>All cases (145)</i>	<i>All ON (51) compared with all controls (139)</i>	<i>All IDLs (36)</i>	<i>All CD MS (62)</i>	<i>All DR2+ cases (70) vs all DR2+ controls (63)</i>	<i>All DR3+ cases (31) vs all DR3+ controls (37)</i>	<i>All DR2/3- cases (44) vs all DR2/3- controls (39)</i>
No history of rubella (cases/controls)	0.90 (65/69)	0.83 (21/69)	1.01 (18/69)	0.84 (26/69)	0.78 (32/37)	0.72 (12/20)	2.01 (21/12)
Rubella aged ≤ 6 yrs (cases/controls)	0.70 (27/37)	0.74 (10/37)	0.73 (7/37)	0.60 (10/37)	0.90 (11/11)	0.98 (9/11)	0.41 (7/15)
Rubella aged > 6-11 yrs (cases/controls)	1.32 (33/24)	1.36 (12/24)	1.29 (8/24)	1.21 (13/24)	1.7 (17/9)	1.19 (5/5)	0.97 (11/10)
Rubella aged > 11 yrs (cases/controls)	2.13 (20/9)	2.42 (8/9)	1.29 (3/9)	2.24 (9/9)	1.5 (10/6)	5.97 (5/1)	2.87 (5/2)
<i>P</i> value	0.02	0.09			n.s.	n.s.	0.07

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance. ON = optic neuritis; IDL = isolated demyelinating lesions; CD MS = clinically definite MS.

provide a fourth group of cases and controls. The *P* values quoted in the text and tables relate to the χ^2 , Kruskal Wallis or Mann-Whitney tests and not these relative risks, although each is derived from the same observations. The analysis of age of infection is based only on those reporting a particular illness since in most instances, serological tests showed that individuals without this history had been exposed at some unknown time; relative risks have, nevertheless, been calculated for this group.

All cases were compared with all controls and a comparison was then made between individuals with optic neuritis, isolated demyelinating lesions, CD MS in relapse, other neurological diseases and normal controls. These were each repeated in analyses restricted to DR2+, DR3+ and DR2/3- individuals. Lastly, the results were compared between all DR2+, DR3+ or DR2/3- individuals irrespective of clinical classification. We have made multiple comparisons and 8 significant differences ($P \leq 0.05$), would be expected by chance; if stringent statistical corrections are applied to the individual *P* values, none of our results remains significant. But since our purpose was to confirm previous findings of higher antibody titres and older age of some infections in individuals who develop MS, and to investigate whether these differences are a reflection of genetic susceptibility using well validated HLA markers, we have presented the results with their uncorrected *P* values.

RESULTS

Rubella

163/297 (55%) individuals in whom the history was known reported clinical infection by rubella, but there were no significant differences in frequency between groups. An estimate of the age of infection was obtained in 150/163 (92%) individuals who gave a history of rubella; this occurred at a significantly later age in all patients with demyelination compared with all controls ($P < 0.02$; Table 3). Older age of infection was observed in each category of patients with demyelination compared with either control group ($P < 0.09$; Table 3). No significant difference in age of infection was found between cases and controls in analyses confined to DR2+, DR3+ or DR2/3- individuals, although in each case the relative risk for developing MS increased with age of infection.

TABLE 4. RELATIVE RISKS FOR DEVELOPING DEMYELINATION.
RUBELLA ANTIBODY TITRE

	<i>All cases</i> (176)	<i>All ON (64)</i> <i>compared with all controls (160)</i>	<i>All IDLs</i> (43)	<i>All CD MS</i> (69)	<i>All DR2+ cases (89)</i> <i>compared with all DR2+ controls (77)</i>	<i>DR2+ ON</i> (29)	<i>DR2+ IDLs</i> (23)	<i>DR2+ CD MS</i> (37)
No rubella antibody (cases/controls)	0.75 (14/17)	0.73 (5/17)	0.22 (1/17)	1.09 (8/17)	0.55 (7/11)	0.48 (2/11)	0.30 (1/11)	0.76 (4/11)
Rubella titre < 125 (cases/controls)	0.66 (57/73)	0.62 (18/73)	0.76 (15/73)	0.63 (20/73)	0.57 (23/35)	0.45 (6/35)	0.48 (5/35)	0.71 (12/35)
Rubella titre 126-140 (cases/controls)	1.20 (61/46)	1.30 (24/46)	1.13 (14/46)	1.16 (23/46)	1.17 (31/23)	1.27 (11/23)	1.16 (8/23)	1.08 (12/23)
Rubella titre > 140 (cases/controls)	1.82 (48/24)	1.77 (17/24)	2.01 (13/24)	1.74 (18/24)	3.03 (28/8)	3.32 (10/8)	3.77 (9/8)	2.34 (9/8)
<i>P</i> value	0.001	0.003			0.0007	0.001		

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance.

All cases (176)

Rubella antibody was present in 305/336 (91 %) individuals and there were no significant differences in frequency of detection between groups. Antibody titres were higher in all patients with demyelination than all controls ($P < 0.001$; Table 4). Higher antibody titres were present in patients with isolated demyelinating lesions or optic neuritis than in those with CD MS in relapse, and in each of these groups of patients with demyelination than in either control population ($P < 0.003$; Table 4). The overall results could be entirely attributed to the difference in rubella antibody titre between all DR2+ cases and DR2+ controls ($P < 0.0007$; Table 4). In this genetic group, rubella antibody titres were also higher in patients with optic neuritis or isolated demyelinating lesions than in those with CD MS in relapse and in each of these groups than in either control population ($P < 0.001$; Table 4). No difference in rubella titre was seen in analyses confined to DR3+ or DR2/3- cases and controls, considered collectively or in the separate groups.

Measles

290/322 (90%) individuals in whom the history was known reported clinical infection by measles, but no significant differences were observed between groups. The estimated age of infection was obtained in 284/290 (98 %) individuals who gave a history of measles; this occurred at a significantly higher age in all cases than controls ($P < 0.05$; Table 5) but there was no difference between patients in each of the three categories of demyelination. The result obtained by comparing all cases with controls could be attributed to the difference between all DR2+ cases and controls ($P < 0.05$; Table 5); a similar trend was observed in the analysis restricted to DR3+ but not DR2/3- individuals (Table 5).

Complement fixation test (CFT) and haemagglutination inhibition (HAI)

TABLE 5. RELATIVE RISKS FOR DEVELOPING DEMYELINATION.
AGE OF MEASLES INFECTION

	<i>All cases (163)</i>	<i>All ON (59) compared with all controls (153)</i>	<i>All IDLs (40)</i>	<i>All CD MS (64)</i>	<i>All DR2+ cases (80) vs all DR2+ controls (70)</i>	<i>All DR3+ cases (37) vs all DR3+ controls (37)</i>	<i>All DR2/3- cases (46) vs all DR2/3- controls (46)</i>
No history of measles (cases/controls)	1.37 (19/13)	1.20 (6/13)	2.06 (7/13)	1.10 (6/13)	1.46 (10/6)	0.5 (3/6)	6.0 (6/1)
Measles infection aged ≤ 4 yrs (cases/controls)	0.75 (48/60)	0.86 (20/60)	0.57 (9/60)	0.76 (19/60)	0.60 (20/29)	1.0 (11/11)	0.85 (17/20)
Measles infection aged > 4-6 yrs (cases/controls)	1.0 (49/46)	1.07 (19/46)	1.08 (13/46)	0.88 (17/46)	1.15 (25/19)	0.85 (11/13)	0.93 (13/14)
Measles infection aged > 6 yrs (cases/controls)	1.3 (47/34)	1.07 (14/34)	1.24 (11/34)	1.55 (22/34)	1.37 (25/16)	1.7 (12/7)	0.91 (10/11)
<i>P</i> value	0.05	n.s.			0.05	n.s.	n.s.

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance

TABLE 6. RELATIVE RISKS FOR DEVELOPING DEMYELINATION.
MEASLES HAEMAGGLUTINATION INHIBITION ANTIBODY TITRE

	<i>ALL cases (176)</i>	<i>All ON (64) compared with all controls (159)</i>	<i>All IDLs (43)</i>	<i>All CD MS (69)</i>	<i>All DR2+ cases (89)</i>	<i>DR2+ ON (29) compared with all DR2+ controls (76)</i>	<i>DR2+ IDLs (23)</i>	<i>DR2+ CD MS (37)</i>
No measles antibody (cases/controls)	2.71 (3/1)	— (0/1)	— (0/1)	6.9 (3/1)	1.71 (2/1)	— (0/1)	— (0/1)	4.11 (2/1)
Measles titre 1: 8-64 (cases/controls)	1.03 (40/35)	0.57 (8/35)	1.58 (15/35)	1.12 (17/35)	0.95 (20/18)	0.58 (4/18)	1.83 (10/18)	0.68 (6/18)
Measles titre 1: 128 (cases/controls)	0.75 (50/60)	0.83 (20/60)	0.74 (12/60)	0.69 (18/60)	0.65 (23/30)	0.97 (10/30)	0.66 (6/30)	0.48 (7/30)
Measles titre ≥ 1:256 (cases/controls)	1.19 (83/63)	1.42 (36/63)	0.94 (16/63)	1.13 (31/63)	1.39 (44/27)	1.45 (15/27)	0.86 (7/27)	1.67 (22/27)
<i>P</i> value	n.s.	0.04			n.s.	0.04		

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance

measles antibody was detected in serum from 279/336 (83%) and 331/336 (98%) individuals, respectively. No significant difference in frequency of detection or titre of CFT antibody was found between groups. There were no differences in HAI measles antibody titre between all cases and all controls, but higher titres were present in patients with optic neuritis than other categories of demyelination or controls in analyses of all ($P < 0.04$) and DR2+ ($P < 0.04$; Table 6) individuals.

Mumps

202/320 (63%) individuals in whom the history was known reported mumps infection. There was a higher reported frequency of mumps in all cases (118/168,

TABLE 7. RELATIVE RISKS FOR DEVELOPING DEMYELINATION.
AGE OF MUMPS INFECTION

	<i>All cases (163) vs all controls (151)</i>	<i>All DR2+ cases (80)</i>	<i>DR2+ ON (26) compared with all DR2+ controls (69)</i>	<i>DR2+ IDLs (21)</i>	<i>DR2+ CD MS (33)</i>	<i>All DR3+ cases (35) vs all DR3+ controls (36)</i>	<i>All DR2/3- cases (48) vs all DR2/3- controls (46)</i>
No history of mumps (cases/controls)	0.68 (50/68)	0.72 (25/30)	0.80 (9/30)	0.55 (5/30)	0.77 (11/30)	0.51 (9/18)	0.77 (16/20)
Mumps infection ≤ 5 yrs (cases/controls)	1.05 (42/37)	0.65 (19/25)	0.64 (6/25)	0.92 (7/25)	0.50 (6/25)	2.57 (10/4)	1.56 (13/8)
Mumps infection 5-8 yrs (cases/controls)	1.36 (41/28)	1.65 (21/11)	1.45 (6/11)	2.39 (8/11)	1.33 (7/11)	0.80 (7/9)	1.56 (13/8)
Mumps infection > 8 yrs (cases/controls)	1.54 (30/18)	4.3 (15/3)	4.42 (5/3)	1.09 (1/3)	6.27 (9/3)	1.85 (9/5)	0.57 (6/10)
<i>P</i> value	n.s.	0.001	0.01			n.s.	n.s.

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance

70%) than all controls (84/152, 55 %; $P < 0.008$). Mumps infection occurred more frequently in all patients with optic neuritis (41/59, 68 %), isolated demyelinating lesions (32/42, 76 %), CD MS in relapse (45/66, 68 %) and patients with other neurological diseases (36/56, 64 %) than in normal controls (48/96, 50 %; $P < 0.02$). There was a higher frequency of mumps infection in DR3+ cases (27/36, 75 %) compared with DR3+ controls (18/36, 50 %; $P < 0.05$).

No overall difference in age of mumps infection was observed between cases and controls but there was a significantly higher estimated age of infection in DR2+ cases than controls ($P < 0.001$; Table 7); in this genetic group, older age of infection was reported in patients with optic neuritis, isolated demyelinating lesions and CD MS in relapse than in either control population ($P < 0.01$; Table 7). Estimated age of mumps infection did not differ between DR2+, DR3+ or DR2/3- cases although there was a trend towards older age of infection in DR2+ patients with demyelination overall. But DR2+ controls had a significantly lower age of infection than DR3+ or DR2/3- controls ($P < 0.006$; not shown in Tables) and this accounted for the difference observed between DR2+ cases and controls. The presence of mumps antibody titre did not differ between groups.

Parainfluenza I

Parainfluenza I antibody was detected in sera from 327/335 (98 %) individuals and no differences in frequency of detection were found between groups. Antibody titres were higher in DR2+ patients with optic neuritis than those with isolated lesions, CD MS in relapse or either control group ($P < 0.04$; Table 8).

TABLE 8. RELATIVE RISKS FOR DEVELOPING DEMYELINATION.
PARAINFLUENZA I ANTIBODY TITRE

	<i>All cases (176) vs all controls (159)</i>	<i>All DR2+ cases (89)</i>	<i>DR2+ ON (29) compared with all DR2+ controls (77)</i>	<i>DR2+ IDLs (23) vs all DR2+ controls (77)</i>	<i>DR2+ CD MS (37) vs all DR2+ controls (77)</i>	<i>All DR3+ cases (38) vs all DR3+ controls (33)</i>	<i>All DR2/3- cases (49) vs all DR2/3- controls (49)</i>
No para I antibody (cases/controls)	0.90 (4/4)	1.73 (2/1)	— (0/1)	— (0/1)	4.16 (2/1)	0.58 (2/3)	— (0/0)
Para I titre 1-8-32 (cases/controls)	0.85 (60/64)	0.69 (27/34)	0.56 (7/34)	0.89 (9/34)	0.67 (11/34)	0.94 (13/12)	1.11 (20/18)
Para I titre 1-64 (cases/controls)	1.02 (60/53)	1.06 (33/27)	0.69 (7/27)	1.11 (9/27)	1.31 (17/27)	0.67 (7/9)	1.18 (20/17)
Para I titre $\geq 1:128$ (cases/controls)	1.24 (52/38)	1.56 (27/15)	2.65 (15/15)	1.11 (5/15)	1.11 (8/15)	1.54 (16/9)	0.64 (9/14)
<i>P</i> value	n.s.	0.09	0.04			n.s.	n.s.

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance.

TABLE 9. RELATIVE RISKS FOR DEVELOPING DEMYELINATION.
EPSTEIN BARR VIRUS (EBV) ANTIBODY

	<i>All cases (176) vs all controls (160)</i>	<i>All DR2+ cases (89) vs all DR2+ controls (77)</i>	<i>All DR3+ cases (38) vs all DR3+ controls (34)</i>	<i>All DR2/3- cases (49) vs all DR2/3- controls (49)</i>
No EBV antibody (cases/controls)	0.87 (42/44)	0.59 (17/25)	1.79 (12/6)	1.0 (13/13)
EBV titre 1-8 (cases/controls)	1.01 (39/35)	0.97 (19/17)	0.77 (6/7)	1.27 (14/11)
EBV titre 1-16 (cases/controls)	1.02 (46/41)	1.44 (25/15)	0.82 (11/12)	0.71 (10/14)
EBV titre $\geq 1:32$ (cases/controls)	1.09 (49/40)	1.21 (28/20)	0.89 (9/9)	1.09 (12/11)
<i>P</i> value	n.s.	0.06	n.s.	n.s.

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance.

Epstein Barr Virus

EB virus antibody was detected in sera from 250/336 (74 %) individuals but there was no difference in frequency of detection between groups; antibody titres were higher in DR2+ cases than controls ($P < 0.06$; Table 9).

Influenza A

Influenza A antibody was present in serum from 267/336 (79 %) individuals; there was no difference in frequency of detection between all cases and controls, but fewer patients with optic neuritis (43/64, 67 %) had influenza A antibodies present in serum than those with isolated lesions (38/43, 88 %), CD MS in relapse (58/69, 85 %), other neurological diseases (50/65, 77 %) or normal controls (79/97, 81 %; $P < 0.04$; Table 10). This result was due to the lower frequency of detecting influenza A antibody in DR2+ patients with optic neuritis (17/29, 59 %) compared with those with isolated demyelinating lesions (22/23, 96 %), CD MS (32/37, 86 %), other neurological diseases (16/20, 80 %) and normal controls (46/57, 81 %; $P < 0.01$; Table 10).

TABLE 10. NUMBER OF INDIVIDUALS WITH INFLUENZA A ANTIBODY

	<i>All cases</i>	<i>DR2+</i>	<i>DR3+</i>	<i>DR2/3-</i>
Optic neuritis	43/64 (67 %)	17/29 (59 %)	10/15 (67 %)	16/20 (80 %)
Isolated demyelinating lesions	38/43 (88 %)	22/23 (96 %)	6/6 (100 %)	10/14 (71 %)
CD MS in relapse	59/69 (85 %)	32/37 (86 %)	13/17 (76 %)	14/15 (93 %)
Other neurological diseases	49/64 (77 %)	16/20 (80 %)	13/15 (87 %)	20/29 (69 %)
Normal controls	78/96 (81 %)	46/57 (81 %)	16/19 (84 %)	16/20 (80 %)
<i>P</i> value	0.04	0.01	n.s.	n.s.

P values are uncorrected for multiple comparisons and based on χ^2 test.

TABLE 11. RELATIVE RISKS FOR DEVELOPING DEMYELINATION INFLUENZA B ANTIBODY

	<i>All cases (176) vs all controls (160)</i>	<i>All DR2+ cases (89) vs all DR2+ controls (77)</i>	<i>All DR3+ cases (38) vs all DR3+ controls (34)</i>	<i>All DR2/3- cases (49) vs all DR2/3- controls (49)</i>
No flu B antibody (cases/controls)	1.11 (81/66)	1.34 (45/29)	0.74 (10/12)	1.04 (26/25)
Flu B titre 1 : 8 (cases/controls)	1.14 (50/40)	0.94 (25/23)	2.04 (16/7)	0.90 (9/10)
Flu B titre 1 : 16 (cases/controls)	0.82 (27/30)	0.79 (11/12)	0.89 (9/9)	0.78 (7/9)
Flu B titre > 1 : 16 (cases/controls)	0.68 (18/24)	0.53 (8/13)	0.45 (3/6)	1.4 (7/5)
<i>P</i> value	n.s.	0.05	n.s.	n.s.

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance.

Influenza B

Influenza B antibody was detected in samples from 189/336 (56 %) individuals but no differences were found between groups in any of the 9 analyses. Influenza B antibody titres were lower in DR2+ cases than controls ($P < 0.05$; Table 11) but this did not result in a difference between all cases and controls.

Adenovirus

Antibody to adenovirus was detected in sera from 205/335 (61 %) individuals, but there was no difference in frequency of detection between groups; adenovirus titres were higher in DR2/3- patients with optic neuritis, isolated lesions, CD MS and other neurological diseases than normal controls ($P < 0.05$; Table 12).

Varicella Zoster

VZ antibody was detected in sera from 264/336 (79 %) individuals but there was no difference in frequency of detection between groups. There was a trend towards higher residual titres of VZ antibody in DR2/3- cases than controls ($P < 0.08$) despite a lower reported incidence of herpes zoster infection in these cases ($P < 0.07$); higher antibody titres were present in DR2/3- patients with optic neuritis, isolated demyelinating lesions, CD MS and other neurological diseases than in normal controls ($P < 0.01$; Table 13) but there was no difference in frequency of clinical herpes zoster between these groups.

TABLE 12. RELATIVE RISKS FOR DEVELOPING DEMYELINATION. ADENOVIRUS ANTIBODY

	<i>All cases (176) vs all controls (159)</i>	<i>All DR2+ cases (89) vs all DR2+ controls (77)</i>	<i>All DR3+ cases (38) vs all DR3+ controls (33)</i>	<i>All DR2/3- cases (49) vs all DR2/3- controls (49)</i>	<i>DR2/3- ON (20) compared with all (49) or normal (20) DR2/3- controls</i>	<i>DR2/3- IDLs (14) MS (15)</i>	<i>DR2/3- CD MS (15)</i>
No adenovirus antibody (cases/controls)	0.85 (63/67)	0.77 (32/36)	1.35 (14/9)	0.77 (17/22)	0.67 0.46 (6/22 13)	0.79 0.55 (5/22 13)	0.89 0.61 (6/22 13)
Adenovirus titre 1:8 (cases/controls)	1.24 (33/24)	0.86 (14/14)	1.56 (9/5)	2.0 (10/5)	1.47 1.5 (3/5 2)	1.4 1.43 (2/5 2)	3.27 3.33 (5/5 2)
Adenovirus titre 1:16 (cases/controls)	1.12 (31/25)	1.51 (21/12)	0.58 (4/6)	0.86 (6/7)	0.35 0.33 (1/7 3)	2.0 1.9 (4/7 3)	0.47 0.44 (1/7 3)
Adenovirus titre ≥ 1:32 (cases/controls)	1.03 (49/43)	1.27 (22/15)	0.73 (11/13)	1.07 (16/15)	1.63 5 (10/15 2)	0.70 2.14 (3/15 2)	0.65 2.0 (3/15 2)
<i>P</i> value	n.s.	n.s.	n.s.	n.s.	0.05		

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance.

TABLE 13. RELATIVE RISKS FOR DEVELOPING DEMYELINATION. VARICELLA ZOSTER (VZ) ANTIBODY

	<i>All cases (176) vs all controls (160)</i>	<i>All DR2+ cases (89) vs all DR2+ controls (77)</i>	<i>All DR3+ cases (38) vs all DR3+ controls (34)</i>	<i>All DR2/3- cases (49) vs all DR2/3- controls (49)</i>	<i>DR2/3- ON (20) compared with all (49) or normal (20) DR2/3- controls</i>	<i>DR2/3- IDLs (14) MS (15)</i>	<i>DR2/3- CD MS (15)</i>
No VZ antibody (cases/controls)	0.81 (34/38)	0.92 (17/16)	0.89 (7/7)	0.67 (10/15)	0.65 0.44 (4/15 9)	0.7 0.48 (3/15 9)	0.65 0.44 (3/15 9)
VZ titre 1:8 (cases/controls)	0.91 (33/33)	1.06 (16/13)	1.02 (8/7)	0.69 (9/13)	0.38 0.28 (2/13 7)	1.35 1.02 (5/13 7)	0.50 0.38 (2/13 7)
VZ titre 1:16-32 (cases/controls)	1.24 (67/49)	1.32 (32/21)	1.09 (17/14)	1.28 (18/14)	1.22 2.33 (7/14 3)	1.0 1.9 (4/14 3)	1.63 3.11 (7/14 3)
VZ titre > 1:32 (cases/controls)	0.95 (42/40)	0.77 (24/27)	0.89 (6/6)	1.71 (12/7)	2.45 7 (7/7 1)	1.0 2.86 (2/7 1)	1.4 4.0 (3/7 1)
<i>P</i> value	n.s.	n.s.	n.s.	n.s.	0.01		

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance.

Other Infections and Viral Antibodies

Out of 339 individuals, 122 (36 %) reported an illness, which we judged to have been infective, in the three months before interview but there were no differences between the groups. No significant difference in frequency of clinical infection, antibody detection or titre was found in any comparisons involving the other viruses studied (*see Methods*).

Typhoid Vaccination

Out of 331 individuals in whom the history was known, 91 (27 %) reported vaccination against typhoid fever; there was no difference in reported frequency of vaccination between groups but estimated age of vaccination was higher in all cases than controls ($P < 0.04$; Table 14).

Tetanus Toxoid Vaccination

Out of 333 individuals in whom the history was known, 247 (74 %) reported tetanus toxoid vaccination; fewer DR3+ patients with other neurological diseases

TABLE 14. RELATIVE RISKS FOR DEVELOPING MS. AGE OF TYPHOID FEVER VACCINATION

	<i>All cases (173) vs all controls (158)</i>	<i>All DR2+ cases (87) vs all DR2+ controls (74)</i>	<i>All DR3+ cases (36) vs all DR3+ controls (34)</i>	<i>All DR2/3- cases (50) vs all DR2/3- controls (50)</i>
No history of vaccination (cases/controls)	1.06 (129/111)	1.08 (65/51)	0.98 (25/24)	1.08 (39/36)
Vaccination aged 0-15 yrs (cases/controls)	0.33 (4/11)	0.17 (1/5)	0.31 (1/3)	0.67 (2/3)
Vaccination aged > 15-20 yrs (cases/controls)	0.87 (19/20)	1.17 (11/8)	0.94 (5/5)	0.43 (3/7)
Vaccination aged > 20 yrs (cases/controls)	1.28 (21/15)	0.94 (10/9)	2.36 (5/2)	1.5 (6/4)
<i>P</i> value	0.04	n.s.	n.s.	n.s.

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance.

TABLE 15. NUMBER OF INDIVIDUALS WITH A HISTORY OF TETANUS TOXOID VACCINATION

	<i>All</i>	<i>DR2</i>	<i>DR3</i>	<i>DR2/3-</i>
Optic neuritis	47/64 (73 %)	23/29 (79 %)	9/15 (60 %)	15/20 (75 %)
Isolated demyelinating lesions	36/42 (86 %)	20/22 (91 %)	6/6 (100 %)	10/14 (71 %)
CD MS in relapse	48/68 (71 %)	25/36 (69 %)	13/17 (77 %)	10/15 (67 %)
Other neurological disease	46/63 (73 %)	13/19 (68 %)	5/14 (36 %)	28/30 (93 %)
Normal controls	70/96 (73 %)	41/55 (75 %)	15/21 (71 %)	14/20 (70 %)
<i>P</i> value	n.s.	n.s.	0.04	n.s.

P values are uncorrected for multiple comparisons and based on χ^2 test.

had received this vaccination (5/14, 36 %) than DR3+ patients with optic neuritis (9/15, 60 %, isolated demyelinating lesions (6/6, 100 %), CD MS (13/17, 77 %) or normal controls (15/21, 71 %; $P < 0.04$; Table 15).

Comparisons of the reported frequency and estimated age of all other vaccinations showed no differences between groups.

Exposure to House Pets

There was no difference in reported frequency of dog or cat ownership between groups but duration of exposure to dogs was higher in all cases than controls ($P < 0.03$; Table 16). This could not be attributed to a difference in comparisons between DR2+, DR3+ or DR2/3- cases and controls and there were no significant differences in estimated duration of exposure to dogs between patients in each of the categories of demyelination or either control group.

HLA-DR Groups

There was a trend towards increased incidence of rubella infection in DR2/3- controls (31/43, 72 %) compared with the corresponding group of cases (23/44, 52 %; $P < 0.09$) and in all DR2/3- individuals (62/99, 63 %) compared with the DR2+ (77/160, 48 %) and DR3+ (48/75, 51 %) groups ($P < 0.06$; not shown in Tables). Higher rubella antibody titres were found in all DR2/3- individuals

TABLE 16. RELATIVE RISKS FOR DEVELOPING DEMYELINATION.
DURATION OF EXPOSURE TO DOGS

	<i>All cases (177) vs all controls (164)</i>	<i>All DR2+ cases (89) vs all DR2+ controls (77)</i>	<i>All DR3+ cases (38) vs all DR3+ controls (37)</i>	<i>All DR2/3- cases (50) vs all DR2/3- controls (50)</i>
No history of dog exposure (cases/controls)	1.14 (58/47)	1.23 (27/19)	0.81 (10/12)	1.31 (21/16)
Dog exposure for ≤ 10 yrs (cases/controls)	0.67 (41/57)	0.72 (24/29)	0.49 (5/10)	0.67 (12/18)
Dog exposure for $> 10-25$ yrs (cases/controls)	1.28 (47/34)	1.06 (22/18)	1.82 (15/8)	1.2 (10/8)
Dog exposure for > 25 yrs (cases/controls)	1.10 (31/26)	1.26 (16/11)	1.11 (8/7)	0.88 (7/8)
<i>P</i> value	0.03	n.s.	n.s.	n.s.

P values are uncorrected for multiple comparisons and based on Mann-Whitney U test or Kruskal Wallis one-way analysis of variance.

(109/114, 96 %) compared with the DR2+ (167/188, 89 %) and DR3+ (68/79, 86 %) groups ($P < 0.05$; not shown in Tables). Influenza B antibody titres were higher in DR3+ individuals (54/79, 68%) than in the DR2+ (103/188, 55%) or DR2/3- (56/114, 49 %) groups, irrespective of clinical category ($P < 0.03$; not shown in Tables).

Age and Sex

Several differences were observed between males and females (not shown in Tables) which could have influenced the results. Taken with the findings of higher measles CFT ($P < 0.02$), parainfluenza 2 ($P < 0.004$), parainfluenza 3 ($P < 0.008$) antibody titres and age of typhoid vaccination ($P < 0.06$) in females than males, our bias of female cases will have overestimated the reported differences; but of these, only age of typhoid vaccination was found to be significant ($P < 0.04$). Conversely, older age of tetanus vaccination in females than males ($P < 0.002$) will have under-estimated the difference between cases and controls which was not significant in our study.

DISCUSSION

Serum antibodies against 16 viruses and retrospective details of exposure to childhood and more recent infectious illnesses have been compared in 341 adult patients with demyelination or controls; our two main findings are differences in age of infection and/or antibody titres between patients and controls involving rubella, measles, mumps, influenza A, influenza B, parainfluenza I, Epstein Barr, adeno and varicella zoster viruses. The overall results could in many instances be attributed to differences between DR2+ cases and controls. Antibody titres were not identical in each group of patients although these differences were not significant; higher titres were more often observed in cases with optic neuritis than those with other categories of demyelination or either control population. However,

exceptions to these general findings included increased varicella zoster and adenovirus antibody titres in DR2/3- patients with demyelination which did not result in a difference between all cases and controls; influenza A and B antibody titres were increased in all controls compared with cases. Although some differences were observed between all DR2+, DR3+ and DR2/3- individuals, irrespective of clinical classification, these did not account for any of the results; nor could they be attributed to differences in age or sex distribution between cases and controls. Some of our observations might have arisen by chance and the individual findings should not be overinterpreted, but the results complement previous studies.

Panelius *et al.* (1973) demonstrated a trend towards older age of infection by measles in patients with MS, especially females, compared with sibling or unrelated controls; Alter and Cendrowski (1976) demonstrated an increased incidence of exposure to mumps, rubella, sore throats and in particular, measles between the ages of 5-9 years in MS patients. Poskanzer *et al.* (1980a) reported higher age of infection by measles and mumps in all individuals from their study in the Orkney and Shetland Islands than expected by comparison with other areas of the United Kingdom, but there were no differences between cases and controls, although age of chickenpox infection was lower in their patients. Haile *et al.* (1982) found a higher odds ratio (equivalent to relative risk) for developing MS with mumps infection and more patients than controls had measles aged between 5-9 years. Currier and Eldridge (1982) showed an association between age of childhood infections and age at onset of symptoms due to MS in twins, one in each pair of which was already known to have the disease, but age of infection did not influence concordance for the disease. Sullivan *et al.* (1984) in a case-control study demonstrated older age at infection by measles in familial but not sporadic cases of MS compared with friend controls; patients' mothers reported older age at infection in both categories compared with their friends' mothers; mothers also reported much older age of mumps infection in sporadic and to a lesser extent familial cases of MS.

Estimated age of typhoid but no other vaccinations was higher in our cases than controls. Currier *et al.* (1974) found that fewer inoculations and vaccinations were reported by patients than controls; conversely a severe immunization reaction was recalled more often by patients. Others have found no difference between patients and controls with respect to incidence or age of BCG, smallpox, diphtheria, poliomyelitis, yellow fever, cholera and typhus vaccinations (Beebe *et al.*, 1967; Andersen *et al.*, 1981).

Although we found no difference in incidence of infectious illnesses in the three months before entry in this study, Sibley and Foley (1965) reported that up to 50 per cent of relapses are preceded by clinical infection; more recently, Sibley *et al.* (1985) showed in a prospective study that 27 per cent of relapses were preceded by an infectious illness although overall viral infections were less common in MS patients than controls; relapse rates were three times higher at the time of infection

irrespective of severity of disease and 8.6 per cent of all viral-like infections were followed by relapse.

In our study, patients with demyelination had an increase in estimated duration of domestic exposure to dogs and reduced cat contact, compared with controls. Subjective assessment of exposure may have been influenced by the hypothesis that contact with dogs and infection by canine distemper are important factors in the aetiology of MS (Cook and Dowling, 1980) which was widely publicized at the time we were interviewing patients. However, we found no difference in reported exposure to dogs between patients with established MS, who may have been familiar with current views on the aetiology of the disease, and cases with isolated demyelination, including optic neuritis, many of whom were unaware of the diagnostic significance of their symptoms. Anderson *et al.* (1984) also reported a reduction in domestic contact with cats in patients with MS; although exposure to dogs was not altered, there was an increased history of canine distemper in a household dog.

Most previous studies have demonstrated higher serum measles antibody titres in MS patients than controls, but no increase in serum titres to rubella, mumps, parainfluenza I, cytomegalovirus CMV, HSV-I, HSV-II, varicella zoster, vaccinia and coronaviruses OC43 or 229E (Johnson *et al.*, 1980; Madden *et al.*, 1981; Salmi *et al.*, 1982; Woyciechowska *et al.*, 1982). Poskanzer *et al.* (1980a) found no difference in antibody titre against 17 viruses including coxsackie B3/4, echo 4/9, poliomyelitis and canine distemper virus, viruses which we did not investigate, in their study from the Orkney and Shetland Islands. There have been fewer such studies in patients with optic neuritis; Link *et al.* (1973) found that measles haemolysin inhibiting antibody titres were higher in serum from patients with optic neuritis who had CSF oligoclonal IgG synthesis than others, whereas no difference was seen in mean serum titre of adenovirus, poliovirus and HAI/CFT measles antibodies, between the groups.

Following the discovery of histocompatibility antigen associations with MS, HLA linked serological abnormalities have been sought especially for measles. But the available studies do not demonstrate conclusively whether the finding of increased serum titres is an effect of HLA type over and above changes attributable to the disease itself, or whether immune reactivity is altered in all individuals possessing the HLA antigens which increase susceptibility to MS (Jersild *et al.*, 1973a; Arnason *et al.*, 1974; Myers *et al.*, 1976; Whitaker *et al.*, 1976; Paty *et al.*, 1977; Poskanzer *et al.*, 1980b; Visscher *et al.*, 1981); and many studies have shown no correlation between HLA antigens and serum viral antibody titres in family studies or in comparisons of unrelated patients and controls so that taken together, no consistent pattern of altered reactivity has emerged from these investigations.

Our study adds to and extends all these observations; although we have studied a larger number of individuals and viruses than previous investigators, and made comparisons in well-defined categories of demyelination with an emphasis on early involvement, using serological reagents capable of detecting all HLA-A, -B, -C

and -DR alleles, standardized by the Seventh International Histocompatibility Workshop, the results must be interpreted with considerable caution. In making multiple comparison it is likely that associations will occur by chance and after applying stringent statistical corrections for this possibility, none of our findings remains significant. Also there are likely to be considerable reporting errors in a retrospective assessment of childhood illnesses, even when the patients' recollections are supplemented by parental information or records of general practitioners. Patients with MS may be better motivated to recall accurately the occurrence and timing of childhood infections than unaffected controls, and methodological factors in a retrospective study could lead to spurious differences in estimating ages of infection. Although a validity survey carried out by Haile *et al.* (1982) identified inaccuracies in the reported age of childhood infections, Alter and Cendrowski (1976) and Haile *et al.* (1982) concluded that the direction and degree of inaccuracy did not differ between cases and controls and had not influenced their results. But we would not regard a particular age as critical for susceptibility to MS and would attach no significance to our findings in the assessment of individual patients. Our method of selecting controls identified large numbers of unrelated individuals, living in the same area as our patients and exposed to the same general environmental conditions, but the method will have selected some controls who had close working contact with each other and this may have influenced the serological findings. Interactions between measles antibody, HLA type and sex have been reported (Fewster *et al.*, 1977), but in many instances the bias in favour of females in our patient population has tended to underestimate the differences between cases and controls. Because of the nature of their symptoms and the referral pattern to our clinics, cases with optic neuritis were studied earlier and often found to have higher antibody titres than those in the other disease categories, perhaps reflecting more recent environmental events and anamnestic responses, but we have no information on serial changes or those related to disease activity.

Although some organisms that we studied have previously been isolated from patients with MS, we have provided no new evidence implicating a specific virus in the aetiology of the disease. Taken with the evidence for increased intrathecal and spontaneous or mitogen-stimulated peripheral blood IgG synthesis (Goust *et al.*, 1982), the fact that significant differences were observed between patients with MS and controls involving several but not all organisms suggests that we are observing one aspect of a relatively nonspecific alteration in immune responsiveness in patients with MS in which there is impaired regulation of B cell function. T suppressor cells influence B lymphocyte responses to many antigens and although there is evidence for reduced T suppressor numbers (Compston, 1983; Hauser *et al.*, 1983) and function (Antel *et al.*, 1979; Tjernlund *et al.*, 1984) in patients with MS, B cell overactivity in peripheral blood cannot be explained only by postulating a defect in T-B suppressor activity; reconstituting T and B cells from normal controls and MS patients in all combinations shows that B cell function in patients

with MS is independent of T cell control (Goust *et al.*, 1982); whatever the immunological explanation for these results, the possibility exists that virus infection itself stimulates these persistent B lymphocyte abnormalities.

MS as presently defined may be the expression of more than one disease and our findings of different patterns of immune reactivity depending on HLA-DR type adds to the evidence for genetic heterogeneity available from population and family HLA studies, in patients with MS (Ho *et al.*, 1982). We did not attempt a retrospective assessment of clinical course, depending on HLA-DR type in the patients with established MS, but several studies suggest that a form of MS exists, characterized by the presence of Dw2/DR2 or linked alleles, early onset and more severe or rapid disability, increased CSF IgG production, abnormal reactivity to a variety of antigens and improved response to treatment (Jersild *et al.*, 1973b; Bertrams *et al.*, 1974; Stendahl-Brodin *et al.*, 1979; Mertin *et al.*, 1982; Dejaegher *et al.*, 1983) with milder disease progression in DR3+ cases (Ilonen *et al.*, 1977; Engell *et al.*, 1982); others have found a positive correlation between the presence of HLA-DR3, disease severity and nonresponsiveness to immunosuppression (Madigand *et al.*, 1982; Delasnerie-Laupretre *et al.*, 1982; Lamoureux *et al.*, 1983).

We do not think it possible to list clinical, epidemiological, immunological and genetic characteristics which can be used in the management of individual patients, but our study does permit a more general conclusion about the relationship of the HLA system to MS. The results, showing that age of infection and antibody titres differ between cases and HLA matched controls, with no difference between all individuals depending on HLA-DR type, indicate that patterns of immune reactivity in patients with demyelination are not merely a consequence of the increased frequency of DR2. Therefore, age at infection, enhanced or persistent antibody responses and presence of specific HLA antigens are probably independent factors associated with an increased risk of MS, but each may be involved in the sequence of events leading to demyelination.

The epidemiological approach, investigating multiplex families and using pedigree analysis, established the concept of genetic susceptibility to MS and demonstrated that inheritance is probably polygenic. HLA phenotype studies have provided supportive laboratory evidence, identified a small area on the sixth human chromosome as uniquely important in determining susceptibility to the disease, confirmed the probable importance of polygenic inheritance and provided evidence for heterogeneity in the pathogenesis. Attempts to identify HLA-linked immunological abnormalities by which to explain the mechanism of these associations have not been successful and we do not think that this approach, using phenotypic markers, will provide further insight into the disease. But HLA phenotype studies have prepared the way for more detailed comparisons between unrelated individuals with MS, or within multiplex MS families, using recombinant DNA techniques with gene probes for the major histocompatibility complex; it is already clear that detection of restriction fragment length polymorphisms can

reveal HLA-D region typing differences between HLA identical individuals, defined by serological or cellular techniques (Cohen *et al.*, 1984).

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