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A 'profile' of immune responsiveness in multiple sclerosis

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SUMMARY

An 'immunological profile' of various indices of B-cell function and T-cell function was developed for the 'early' case of multiple sclerosis (MS). This was compared against two groups of controls comprising age and sex-matched healthy subjects, and patients with other disabling neurological diseases (CNS controls) who were matched for age, sex, and type and duration of disability. Some indices of humoral immune responsiveness, such as the induced primary response to monomeric flagellin and the 'resting' levels of antibody to measles and rubella viruses, showed significant augmentation. Cellular immune deficits were attributed to an illness effect *per se* because (a) cell-mediated immunity was depressed, but only when compared with that of healthy subjects and not when compared with that of the CNS controls, and (b) transformation responses of lymphocytes to viral antigens were inversely related to disability status. The abnormalities in humoral immune responses demonstrable in this study do not provide an explanation for this disease; if there is a relevant 'immunological fault', the nature of this needs to be sought from within the neuraxis rather than from the systemic circulation.

INTRODUCTION

The likeliest explanations of the cause of multiple sclerosis are a predisposition to an infection in childhood and a maladaptive immune response. The infectious cause of multiple sclerosis is favoured by epidemiological data from migrant populations (Dean, 1967; Leibowitz, Kahana & Alter, 1969), the presence of monoclonal immunoglobulin in the cerebrospinal fluid (CSF) and evidence for a transmissible agent in the brain and serum of patients with multiple sclerosis (Carp *et al.*, 1972; Koldovsky *et al.*, 1975). An association between infection with measles virus and multiple sclerosis is suspected because of the repeated finding in blood of elevated titres of antibody to the measles virus (Adams & Imagawa, 1962; Brody, Sever & Edgar, 1972). Studies *in vitro* have purported to show a specific defect of the cell-mediated immune (CMI) response to measles virus antigen, which could either facilitate primary infection or promote latent infection with the measles virus in the central nervous system (Utermohlen & Zabriskie, 1973; Ciongoli *et al.*, 1973), but this claim has been refuted by others (Symington & Mackay, 1977). Experimental evidence for an immunological cause of multiple sclerosis is provided by some resemblances between multiple sclerosis and experimental autoimmune encephalomyelitis (EAE), which is determined by a T cell-mediated immune response against an autoantigen in the brain (Mackay, Carnegie & Coates, 1973; Bernard, Leydon & Mackay, 1976).

A demonstrably abnormal immune response in multiple sclerosis could be a cause or consequence of the disease. Hence fallacious conclusions in studies on multiple sclerosis could be made unless the study included not only healthy control subjects but also those carefully matched for variables such as age, sex, duration, phase and severity of disease and degree of disability. Many studies purporting to show an altered immune function in multiple sclerosis have not fulfilled such criteria.

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The aim of the present study was to develop an immunological 'profile' of the early case of multiple sclerosis, to determine whether there were: (a) general cellular or humoral immune dysfunction intrinsic to multiple sclerosis and independent of the debilitating effect of the disease itself; (b) specific immunological defects or reactivity to various commonly encountered viruses; or (c) reactions to autoantigens, including neural autoantigens.

MATERIALS AND METHODS

Patients. Multiple sclerosis was diagnosed according to the criteria of Rose *et al.* (1968). The neurological status of each patient was evaluated according to the degree of disability and scored from 0 to 10, after the disability scale of Rose *et al.* (1968). Twelve 'early' patients, whose mean duration of symptoms (± standard deviation, s.d.) was 4± 2 years, were selected so as to exclude, as far as possible, the known adverse effects of debilitating disease on immune function (Rowley & Mackay, 1969; Thompson *et al.*, 1975). There were five males and seven females; none had evidence of infection or malnutrition and one only was receiving specific treatment, this being a small dose (7 mg) of prednisolone; the mean age was 38± 8 years and the age range was 28–53 years.

For some studies, the group size was increased to twenty cases by the inclusion of two males and six females, for whom the mean duration of disease was 5 years. These patients were not available for all the tests. The number of patients included in the various studies is shown in Table 1. Except where stated, all assays were performed during a period of clinical remission, and at least 8 weeks after any clinical exacerbation. Additional patients, who were unselected for the duration of disease, were typed for histocompatibility antigens and tested for autoantibodies.

B-lymphocyte profile. Counts of B lymphocytes. Counts of B lymphocytes were made on lymphocytes separated from peripheral blood centrifuged through Ficoll–Isopaque (Böyum, 1968). B lymphocytes were identified by direct immunofluorescence using a polyvalent fluorescein-labelled rabbit anti-human globulin. For absolute counts, the number of peripheral blood lymphocytes was calculated from total and differential blood leucocyte counts.

Immunoglobulins. The level of IgG in the serum was determined by immunodiffusion (Fahey & McKelvey, 1965) and that of IgG in the cerebrospinal fluid was determined by a modification of the electrophoresis technique of Laurell (1966), using antisera supplied by Dako (Denmark) and agarose supplied by Litex (Denmark).

Antibody response to immunization with flagellin. The primary antibody-producing capacity to injected antigen was measured after subcutaneous immunization with 5 μg of monomeric flagellin from *Salmonella adelaide* (Ada, Nossal & Pye, 1964) administered during disease remission. Antibody was assayed before injection, and at 1, 2, 6 and 10 weeks after injection, by a tanned sheep erythrocyte agglutination procedure (Rowley & Mackay, 1969). Total antibody, and antibody remaining after the treatment of serum with 2-mercaptoethanol (ME) for 1 hr at 37°C and considered to be IgG, were determined.

Autoantibodies. Fifty-one patients were tested for autoantibodies to nuclei, gastric parietal cells, smooth muscle, thyroid epithelial cells and mitochondria by indirect immunofluorescence (Whittingham & Mackay, 1969).

Antibody responses to viruses. The humoral immune response to several viruses was measured in paired specimens of serum and CSF. Antibodies to antigens of influenza A and B, parainfluenza 1, measles, herpes simplex, adenovirus, cytomegalovirus and mumps S and V were measured by complement fixation, and antibodies to rubella and vaccinia virus antigens by haemagglutination inhibition.

T-lymphocyte profile. Counts of T lymphocytes. Counts of E rosette-forming cells were made on peripheral blood lymphocytes according to the method of Kaplan & Clark (1974). An E rosette-forming cell was defined as a lymphocyte with three or more adherent sheep erythrocytes and was readily identified by fluorescence after staining with acridine orange (Brostoff, 1974).

Delayed-type hypersensitivity (DTH) responses. Cutaneous responses were tested to five ubiquitous microbial antigens, candidin (Bencard), mumps skin test antigen (Eli Lilly), trichophyton (Bencard), the purified protein derivative of tuberculin (Commonwealth Serum Laboratories, Melbourne) and streptokinase–streptodornase ('varidase') (Lederle). Reactions to these antigens were read at 48 hr and scored as positive when the mean of the maximum and minimum diameters of the induration area was greater than 0·6 cm; the score was the sum of positive reactions (Toh *et al.*, 1973). The capacity to develop sensitization to DNCB was assessed using, initially, test applications to the skin of 2000 μg and 50 μg DNCB and reading the response at 14 days; if the response was negative the subject was rechallenged with 50 μg DNCB and the response read at 48 hr. The procedure and scoring of reactions from 0 to 4 was as described by Catalona *et al.* (1972) and Thompson *et al.* (1975).

Lymphocyte blastogenic responses to phytohaemagglutinin (PHA). Blastogenic transformation of unstimulated lymphocytes and lymphocytes stimulated with 20 μg PHA (Wellcome reagent grade) was measured by the incorporation of tritiated thymidine into replicating lymphocytes. Serial studies were made on twelve patients, there being thirty-one serial determinations, including four during exacerbation. Blood lymphocytes separated on Ficoll–Isopaque were set up as triplicate cultures of 10^6 lymphocytes in 1 ml Eagle's minimal essential medium supplemented with either 10% foetal calf serum (FCS) (Toh *et al.*, 1973) or autologous serum; the duration of culture was 72 hr and at 66 hr 1 μC tritiated thymidine was added.

Lymphocyte blastogenic responses to viruses. Lymphocyte reactivity to measles (Enders vaccine strain 10^8 particles per ml),

TABLE 1. Patients with multiple sclerosis and controls studied immunologically

Groups	Tests performed	(No. of cases)
Multiple sclerosis		
'Early' cases*	All	(12)
Additional 'early' cases†	Viral antibodies	(20)
	DTH responses	(20)
Additional cases in all stages of MS	Autoantibodies	(51)
	HLA	(40)
Controls		
Healthy subjects (matched with 'early' cases)	All	(12)
Additional healthy subjects	Viral antibodies	(50)
	DTH responses	(32)
	Blastogenic responses to PHA	(20)
Patients with neurological diseases	Viral antibodies	(20)
	DTH responses	(20)
Random population	Autoantibodies	(51)
	HLA	(375)

* Mean duration of symptoms 4 years.
† Mean duration of symptoms 5 years.

parainfluenza (mixture of types 1, 2, 3, 4a, 4b, SV5 and Sendai, $1{\cdot}2\times 10^9$ particles per ml) and vaccinia viruses (2×10^6 particles per ml) was assessed by lymphocyte transformation under conditions similar to those described for PHA with 10% autologous serum in the medium. Measles and parainfluenza viruses were grown in monkey kidney culture and vaccinia (CSL vaccine strain) was grown on chick chorioallantoic membrane (Symington & Mackay, 1977). 'Control' antigens were prepared similarly from uninfected cultures. All viruses were inactivated by β-propiolactone. Dose–response studies were performed with each virus preparation to ascertain the optimal dilutions of antigen. Maximal transformation for measles and parainfluenza viruses was obtained with undiluted preparations, and for vaccinia antigen with a 1:10 dilution.

Lymphocyte blastogenic responses to brain antigens. The brain antigens used in these studies included basic protein of myelin (BPM), prepared from human brain as described by Dunkley & Carnegie (1974), and myelin and synaptosomes, both prepared from the brain of a patient with multiple sclerosis by procedures described by Whittaker (1966). Serial studies were made on twelve patients. Lymphocyte transformation was assessed as described for PHA. The doses of BPM were 5, 10 and 50 μg/ml of culture medium, and cultures were supplemented with either 10% FCS or 10% autologous serum; twenty-nine assays were performed, including four during exacerbation. Human synaptosomes were used in concentrations up to 1000 μg wet weight per ml and myelin in concentrations up to 5000 μg/ml, and cultures were supplemented with autologous serum.

The following procedure was developed as a positive control for the test system. Three rabbits were immunized on four occasions with bovine BPM, twice with 2 mg in Freund's complete adjuvant (FCA) supplemented with 5 mg/ml of *M. tuberculosis* and twice with 0·2 mg in Freund's incomplete adjuvant. All developed severe EAE within 4 weeks. After the first immunization the rabbits were tested weekly for transformation of blood lymphocytes to human BPM, using conditions similar to those used for testing human lymphocytes.

Histocompatibility typing. Forty patients and 375 blood donors were typed using a standard microlymphocytotoxicity test (Mittal *et al.*, 1968) and antisera with well-defined specificities.

Controls. The various control groups are shown in Table 1. The neurological disease in controls (CNS controls) was comparable in degree and duration of disability with that in the patients with multiple sclerosis. The diagnoses for the CNS diseases included pituitary tumour, Alzheimer's disease, Huntington's chorea, past cerebrovascular accident, temporal lobe epilepsy, Parkinson's disease, syringomyelia, cerebral hamartoma, acoustic neuroma, pinealoma and cerebral metastatic tumour. Additional groups of subjects drawn from population surveys were used as controls for autoantibodies (Hooper *et al.*, 1972) and HLA typing; those tested for autoantibodies were age- and sex-matched with the patients with multiple sclerosis.

Statistical tests. The Student's *t*-test was used to test for significant differences between patient and control groups for the mean values of the blood lymphocyte count, B-lymphocyte count, T-lymphocyte count, lymphocyte transformation responses and the logarithm of the titres of antibody to flagellin and various viruses. The Mann–Whitney rank test was used for comparisons of CSF IgG levels. Differences between the two groups in frequencies of autoantibodies and histocompatibility antigens, and scores for DTH reactions, were tested for significance by the χ^2 test with Yates continuity correction: where any expected frequencies were less than 5, an exact test based on the hypergeometric distribution was used. The significances of the correlations of the disability status score (*vide supra*) with lymphocyte transformation response and with the logarithm

of the titre of antibody to measles and rubella viruses were obtained from the Pearson product–moment correlation coefficients.

All significance levels are given as one-tailed probabilities. Where the likely direction of the group differences was not known *a priori*, a two-tailed probability (double the value quoted) was appropriate.

RESULTS

B-lymphocyte profile

Counts of B lymphocytes. The absolute numbers and proportions of B lymphocytes in the blood were similar in patients and age- and sex-matched healthy controls (Table 2).

IgG. The mean serum IgG level was similar in patients with multiple sclerosis and CNS controls. The mean CSF IgG of 0·057 g/l was significantly higher in patients with multiple sclerosis than the level of 0·039 g/l in CNS controls.

Antibody responses to immunization with flagellin. The geometric mean titres of immune antibody (total and IgG) at various time points after primary immunization were significantly elevated in patients with multiple sclerosis compared with healthy controls (Fig. 1).

Autoantibodies. There was an increased frequency of antibody to nuclei of polymorphonuclear leucocytes, but not to any other of the autoantigens tested for in the patients with multiple sclerosis (Table 1).

Antibody responses to viruses. Geometric mean titres of serum antibody to certain viruses were significantly increased in patients with multiple sclerosis. When compared with healthy controls (and also with CNS controls) patients with multiple sclerosis had highly significantly increased titres to rubella virus ($P<0·002$) and measles virus ($P<0·001$). There was no significant correlation between serum levels of measles antibody and the score for disability status (cf. data for cell-mediated immune response to measles). Differences were not significant between the mean titres of antibody in the CSF to any of the viruses when patients with multiple sclerosis were compared with CNS controls.

T-lymphocyte profile

Counts of T lymphocytes. The absolute numbers and proportions of T lymphocytes in the blood were similar in patients and healthy controls (Table 2).

Delayed type hypersensitivity responses. As judged from previous studies (Thompson *et al.*, 1975), healthy subjects give a score of 2 or greater in the test for DTH responses to ubiquitous antigens and to DNCB. In the DTH responses to ubiquitous antigens, the mean score for the twenty patients with multiple sclerosis was 2·5 and was similar to that for twenty patients with other chronic neurological diseases (2·4), but contrasted with that for twenty healthy persons (3·4). A score of less than 2 was given by five of the twenty patients with multiple sclerosis and four of the twenty controls with chronic neurological diseases. The proportion of positive responders in the multiple sclerosis group was significantly less ($P<0·05$) than that in healthy controls, all of whom gave a score of 2 or greater. When the frequencies of response to each of the five antigens were compared singly, there was no significant difference between the multiple sclerosis and neurological disease control groups. In the sixteen subjects with multiple sclerosis tested for sensitization to DNCB, there were two non-responders and the mean score $\pm$s.d. was $2·9\pm1·2$; in the sixteen controls with a disease of the nervous system, there were also two non-responders, and the mean score of $2·9\pm1·1$ was the same.

Lymphocyte transformation responses to PHA. With culture medium supplemented with FCS, the transformation response to PHA, expressed as the stimulation index$\pm$s.d., was $33·2\pm23·2$ for the multiple sclerosis group and $34·4\pm21·9$ for healthy subjects. With the culture medium supplemented with autologous serum, the response to PHA was enhanced, being $55·4\pm54·3$ for the multiple sclerosis group and not significantly greater, $118·9\pm126·0$, for the healthy controls. There was no significant correlation between PHA responses and the score for disability status. No significant differences in stimulation indices were demonstrable between PHA responses tested during exacerbations and remissions.

Lymphocyte transformation response to viruses. In twelve patients studied, the mean stimulation index for measles antigen was $3·8\pm3·1$, and for parainfluenza antigen $3·6\pm3·2$; these were not significantly

TABLE 2. Results of immunological tests in patients with multiple sclerosis and controls. Values recorded as means ± one standard deviation (s.d.)

Test	Multiple sclerosis	CNS controls	P*	Healthy controls	P*
B-cell function					
B-lymphocyte counts (per mm³)	587 ± 302 (28%)	n.t.		700 ± 350 (33%)	n.s.
Immunologublin G (g/l)					
Serum	13·0	13·6		7–19	n.s.
CSF	0·057	0·039		n.t.	< 0·05
Antibody response to flagellin	Increased (see Fig. 1)	n.t.		(see Fig. 1)	
Autoantibodies (%)					
Lymphocyte nuclei	20	n.t.		8	n.s.
Polymorphonuclear nuclei	33	n.t.		6	< 0·002
Gastric parietal cells	10	n.t.		8	n.s.
Thyroid epithelial cell	10	n.t.		12	n.s.
Smooth muscle	2	n.t.		2	n.s.
Mitochondria	0	n.t.		0	n.s.
Viral antibodies (log_{10} titre)					
Influenza A	0·83 ± 0·27	0·84 ± 0·39	n.s.	1·07 ± 0·28	< 0·005
B	0·60 ± 0·31	0·75 ± 0·44	n.s.	0·92 ± 0·43	< 0·005
Rubella	2·54 ± 0·70	2·09 ± 0·77	< 0·05	1·99 ± 0·67	< 0·002
Measles	2·04 ± 0·54	1·68 ± 0·63	< 0·05	1·41 ± 0·38	< 0·001
Herpes simplex	1·35 ± 0·77	1·03 ± 0·51	n.s.	1·40 ± 0·65	n.s.
Adenovirus	0·75 ± 0·38	0·65 ± 0·50	n.s.	0·84 ± 0·55	n.s.
Cytomegalovirus	0·63 ± 0·53	1·05 ± 0·67	< 0·02	0·81 ± 0·61	n.s.
Mumps S	0·84 ± 0·48	0·80 ± 0·50	n.s.	0·59 ± 0·33	< 0·02
V	0·95 ± 0·50	0·97 ± 0·57	n.s.	0·66 ± 0·40	< 0·02
parainfluenza 1	1·03 ± 0·22	0·78 ± 0·35	< 0·05	n.t.	
vaccinia	0·54 ± 0·18	0·58 ± 0·25	n.s.	n.t.	
T-cell function					
T-lymphocyte counts (per mm³)	1465 ± 527 (70%)	n.t.		1495 ± 513 (69%)	n.s.
Delayed hypersensitivity, mean score					
Five antigens	2·5	2·4	n.s.	3·4	< 0·05
DNCB	2·9	2·9	n.s.	n.t.	
Lymphocyte blastogenic response to PHA (S.I.)					
20 μg with FCS	33·2 ± 23·2	n.t.		34·4 ± 21·9	n.s.
20 μg with autologous serum	55·4 ± 54·3	n.t.		118·9 ± 126·6	n.s.
Viral antigens					
Measles	3·8 ± 3·1	n.t.		4·9 ± 5·4	n.s.
Parainfluenza	3·6 ± 3·2	n.t.		3·1 ± 2·5	n.s.
Vaccinia-vaccinated	1·2 ± 0·5	n.t.		2·0 ± 1·3	n.s.
Vaccinia-non-vaccinated	2·9 ± 2·2	n.t.		7·0 ± 5·4	n.s.
Neural antigens (S.I.)†					
5 μg myelin basic protein	0·9 ± 0·34	1·00 ± 0·03	n.s.	0·98 ± 0·10	n.s.
1000 μg myelin	1·09 ± 0·25	1·03 ± 0·07	n.s.	1·02 ± 0·07	n.s.
100 μg synaptosomes	0·90 ± 0·21	0·98 ± 0·10	n.s.	0·95 ± 0·09	n.s.
HLA (%)					
A3	40	n.t.		24	< 0·05
B7	45	n.t.		25	< 0·025

S.I., mean stimulation index; n.t., not tested; n.s., no statistically significant difference.

* One-tailed test. † Culture medium contained FCS.

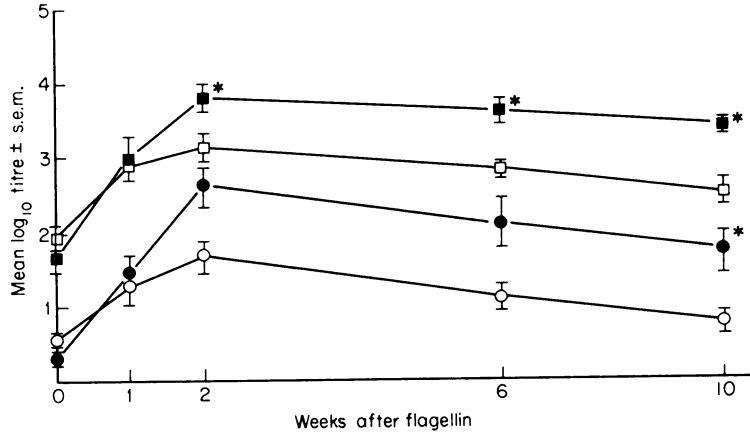

FIG. 1. The humoral immune response to injection of 5 μg flagellin from *Salmonella adelaide* was significantly higher in patients with multiple sclerosis (●, ■) than in healthy matched controls (○, □). (■, □) Total immune response; (○, ●) IgG response; * $P < 0{\cdot}005$.

different from the corresponding responses in healthy subjects, $4{\cdot}9 \pm 5{\cdot}4$ and $3{\cdot}1 \pm 2{\cdot}5$ respectively. The mean stimulation indices for vaccinia virus were $1{\cdot}2 \pm 0{\cdot}5$ in the unvaccinated patients with multiple sclerosis and $2{\cdot}9 \pm 2{\cdot}2$ in the vaccinated patients, and in healthy subjects the corresponding indices were $2{\cdot}0 \pm 1{\cdot}3$ and $7{\cdot}0 \pm 5{\cdot}4$; these differences were not significant. There was a significant inverse correlation between disability scores and the transformation response to measles antigen ($P < 0{\cdot}02$; Pearson correlation coefficient $-0{\cdot}62$), and to vaccinia antigen ($P < 0{\cdot}03$; Pearson correlation coefficient $-0{\cdot}65$), but this did not hold for parainfluenza antigen; these data have been presented in detail by Symington & Mackay (1977).

Lymphocyte blastogenic responses to brain antigens. There was no blastogenic response of blood lymphocytes to BPM in concentrations of 5, 10 and 50 μg/ml of 10^6 cultured cells in any of the twelve patients studied, during remission or exacerbation. Also, no transformation responses were demonstrable when synaptosomes or whole myelin were used as stimulators at various concentrations. A positive control for this system was provided by results from rabbits immunized with BPM in FCA; the transformation responses by peripheral blood lymphocytes to BPM were dose- and time-dependent and uniformly positive with stimulation indices reaching 135, indicating that our procedure would detect BPM-sensitized lymphocytes in human peripheral blood if such were present.

HLA phenotypes. In multiple sclerosis the prevalence for HLA-A3 was 40% *vs* 24% in controls ($P < 0{\cdot}05$), and for HLA-B7 45% *vs* 25% in controls ($P < 0{\cdot}02$); these differences did not remain significant after allowance was made for the number of comparisons. There was no correlation between the HLA phenotype B7 and the level of antibody to measles antigen, or the presence or degree of cell-mediated immune response to measles antigen.

DISCUSSION

There have been many studies on different aspects of immune function in multiple sclerosis, but in few has there been an attempt to examine immunocompetence comprehensively, with the testing of selected patients with 'early' disease and the inclusion of control groups representing healthy subjects and patients with non-demyelinating disease of the central nervous system. The immunological 'profile' in early multiple sclerosis was represented by a humoral immune hyperresponsiveness to certain antigens, including flagellin and antigens of measles and rubella, and a cellular immune hyporesponsiveness attributed to disabling effects of the disease itself.

In regard to the B lymphocyte system, there were normal counts of B cells in the blood, as described in other studies (Nowak & Wajgt, 1975; Reddy & Goh, 1976), and normal levels of immunoglobulins in

the serum; however, levels of IgG in the cerebrospinal fluid were increased. Studies on antibody production showed hyperresponsiveness to a primary immunogenic challenge with a bacterial antigen, flagellin. Unstimulated antibody levels to a range of viral antigens showed a varying pattern, but the most definitive results were the significant increases in mean serum antibody levels to antigens of measles and rubella, in comparison with both groups of controls. There was a decrease in mean levels of influenza antibodies and an increase in mumps antibodies, significantly so in comparison with the healthy controls but not the CNS controls. Hyporesponsiveness of humoral antibody synthesis has been reported in other studies, including a depressed response on primary challenge with the antigens of keyhole limpet haemocyanin (Davis, Hersh & Curtis, 1972) and typhoid vaccine (Field, Green & Miller, 1961), and there were lower unstimulated titres of antibodies to diphtheria and tetanus toxoid antigens, according to Lamoureux *et al.* (1976). It may be, as Lamoureux *et al.* (1976) suggest, that in multiple sclerosis humoral immune responses to various antigens can either be increased or decreased, but we can note that, in the reported studies on decreased humoral immunity, there was no selection for 'early' cases and debility was not taken into account. Previously, we have shown, using immunization with flagellin, that chronic illness *per se* may be associated with a depressed humoral immune response (Lee *et al.*, 1971).

In regard to the T-lymphocyte system, counts of T lymphocytes in peripheral blood were normal, in keeping with other reports (Nowak & Wajgt, 1975; Oger *et al.*, 1975), although Lisak *et al.* (1975) and Allen *et al.* (1976) found that the numbers varied with the clinical activity of the disease. Cutaneous DTH reactivity, using a panel of ubiquitous antigens and DNCB, was depressed in multiple sclerosis when compared with healthy persons, but not when compared with controls with comparable disability due to CNS disease. Johnson & Miller (1963) showed that responses to a panel of microbial antigens were similar to those of normal subjects, and Sever & Kurtzke (1969) reported similarly for cutaneous responses to measles and mumps antigens. Smith, Hughes & Hunter (1961) found an excess of negative tuberculin reactors, and Davis *et al.* (1972) found a depressed DTH response to keyhole limpet haemocyanin; however, the controls in these studies were healthy subjects. We did not find evidence of altered blastogenic responses of lymphocytes, either during remission or exacerbation of disease, but the range of responsiveness was wide. Previous reports on the blastogenic responses of T lymphocytes to the mitogen PHA, reviewed by Lisak (1975), have given varying results and are difficult to assess because the assay conditions differed, and some of the studies were not optimally controlled. We found no differences in blastogenic responses of lymphocytes to the viral antigens of measles, vaccinia and paramyxo viruses, when results were compared with those for healthy age-matched controls; however, the responses to measles and vaccinia viral antigens in multiple sclerosis showed a significant negative correlation with the disability rating. These findings, and our results with DTH and DNCB testing, indicate that the previously reported depression of responsiveness to measles antigen (Utermohlen & Zabriske, 1973; Ciongoli *et al.*, 1973) could be explained by a non-specific depressant effect of illness on T-lymphocyte function, as discussed by Symington & Mackay (1977) and *vide supra*.

T-cell blastogenic responses against the central nervous tissue autoantigens BPM, synaptosomes and whole myelin gave consistently negative results at all stages and phases of multiple sclerosis. Reports claiming positive results with *in vitro* tests for cell-mediated immunity to BPM in multiple sclerosis, using either lymphocyte transformation or leucocyte migration inhibition, are reviewed by Källén, Nilsson and Thelin (1977). Colby *et al.* (1977) reported positive results, using myelin basic A1 protein, with both cell migration inhibition and lymphoblastic transformation assays, most significantly so during exacerbations; our contrasting negative findings gain some added weight because of our use, as a positive control, of blood lymphocytes of rabbits immunized for EAE; such cells underwent vigorous transformation on exposure to BPM. Thus a consensus of opinion must be awaited as to whether there is a disease-specific sensitization of lymphocytes to myelin antigens in multiple sclerosis.

Our present study sought to ascertain whether patients with multiple sclerosis showed immunological differences from healthy subjects during remission or relapse of the disease. Certain differences were demonstrable, notably an augmented B-cell responsiveness to flagellin and viral antigens, and a depressed T-cell responsiveness which, however, was similar to that of matched controls with neurological disease

and, therefore, attributable to an 'illness effect' of the disease itself. Thus whilst the immunological background of the patient predisposed to multiple sclerosis may be slightly different in terms of humoral hyperresponsiveness, this could not provide more than a partial explanation for susceptibility. If importance is given to HLA associations (Jersild *et al.*, 1975; Compston, Batchelor & McDonald, 1976; Opelz *et al.*, 1977) and findings in the CSF of raised levels of immunoglobulins and oligoclonal proteins (Vandvik, Natvig & Wiger, 1976), the immunological 'fault' in multiple sclerosis should be sought for not systemically but in the neuraxis itself, wherein there could be immune stimulation by an antigen which we term the multiple sclerosis-associated antigen (MSAg). The MSAg could be an antigenic determinant of the measles virus, or, more likely, an antigen with some degree of cross-reactivity with measles antigen. The damage in multiple sclerosis could result from the activity of an agent with which the MSAg is associated or from the immune response to the MSAg: either contingency requires identification of the nature of the MSAg. Recently, Levinson, Lisak & Zweiman (1976) have reported techniques applicable to the study of the reactivity of T lymphocytes from CSF to brain antigens such as BPM and oligodendrocytes, and to purified viral antigens. Such techniques could be informative in relation to the immunological events in the brain in multiple sclerosis.

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