PAPERS AND ORIGINALS

Immunological features in multiple sclerosis

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Summary

A clinical and laboratory profile of the immunological system of patients with multiple sclerosis (MS) strongly suggested that many specific immune deficiencies exist in MS. The immunological history showed that patients with MS had had more tonsillectomies, appendicectomies, and childhood infections than matched controls, which suggested that there had been problems in controlling various types of childhood infections. The cell-mediated immune response and the circulating antibody titres were specifically impaired against a variety of antigens. Patients with MS had significantly lower serum antibody titres than controls against many naturally occurring antigens—namely, diphtheria and tetanus toxoids, adenovirus, and mumps viruses. Raised serum antibody titres were found against measles and varicella zoster viruses while no difference was found towards other antigens. The delayed hypersensitivity reaction and the immunological memory of patients with MS were also greatly reduced against the mumps skin test antigens. There were normal amounts of circulating T and B lymphocytes, and the phytohaemagglutinin, concanavallin A, pokeweed mitogen, and encephalitogens lymphocyte transformation was not different from that in controls.

These results indicated that patients with MS have more infectious problems than normal people and that both their T and B cell systems cannot mount a fully normal immunological response to some viral and bacterial antigens, while they give an increased response to others.

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Introduction

The neuropathological lesions in multiple sclerosis (MS) have been linked to an impaired immune mechanism since the discovery of a raised cerebrospinal fluid (CSF) gammaglobulin fraction[1] in which IgG, IgA, and IgM were identified.[2] The presence of an abnormal cell-mediated immune response after intrathecal injection of purified protein derivative of tuberculin (PPD),[3-5] blood cytotoxic lymphocytes, and CSF cytotoxic factor[6] for myelinated nerve cells in tissue culture and the discovery of increased serum and CSF measles antibodies[7] gave more strength to the idea that an immunological defect played a part in the pathogenesis of MS.

Our experiment was designed to evaluate the immunological function of patients with MS using several immunological techniques. Unfortunately, the experiment had to be interrupted because several patients experienced an acute relapse of their symptoms while under investigation.

Patients and methods

The patients studied were all young adults, aged 23-38, who had a combination of the characteristic symptoms of MS, including a history of more than four acute neurological episodes with improvement and objective findings of pyramidal, cerebellar, sensory, and cranial nerve disease. In all cases MS had been clinically diagnosed by neurologists, and the CSF IgG value exceeded 0·035 g/l on repeated analyses. Twenty-three such patients were immunologically investigated when they were not suffering an exacerbation of their disease or being treated with corticotrophin or steroids. Most of them had received treatment at least once in the past. The controls were 23 normal people selected from a pool of about 300 people working in our institute to match the patients in age, sex, and place of origin. Controls and patients answered the same questionnaire and were bled the same day. The normal values for the skin tests were obtained from another group of 104 normal people during another immunological study.[8]

Immunological history—The patients and controls were asked if they had a history of allergies (eczema, hay fever, or asthma), immunological deficiencies, diabetes, or cancer and whether they had ever undergone tonsillectomy or appendicectomy.

Serum and plasma—Blood (60 ml) was withdrawn in BD tubes (Becton-Dickinson Co) from each patient, and 20 ml was put in non-treated tubes and 40 ml in heparinised tubes. Cells were analysed the same day while sera were kept frozen at −20°C until used.

Serum immunoglobulins—Serum IgG, IgA, IgM, IgD, and IgE

levels were determined by radial immunodiffusion.[9] Antisera and standards were from Hyland (USA).

Specific antibodies—Serum viral antibodies against influenza A/Port Chalmers/1/73 (H3N2) designated MRC-10/131c; influenza B/Hong Kong/5/72; adenoviruses (Connaught Canada; 1637-02-7); parainfluenza I and II (Ottawa) and III (Flow 9.234 MC); mumps viral and soluble antigens (Connaught, 1631-01-7); herpes simplex (Ottawa); varicella zoster (VZ Microbiological Associates (MBA) Scott MA-184); cytomegalovirus (MBA: AD-169); and measles (Philadelphia, Vero 76-108) were titrated according to the micro-standardised diagnostic complement fixation technique of the US Department of Health, Education and Welfare. Anti-influenza A (Port Chalmers/1/73 (H3N2) designated MRC-10/131c, and England/42/72 (H3N2) designated MRC-7) and B (Hong Kong/5/72) titres were also measured by the haemagglutination inhibition technique.[10] The antitetanus and antidiphtheria toxoid antibody titres were measured by the passive haemagglutination technique.[11] The influenza A and B and the tetanus and diphtheria toxoids used as antigens for in-vitro testing were the same as those included in the polyvalent vaccine used for assessing immunological memory (see below).

Autoantibodies—The presence of antinuclear factors (ANF) and autoantibodies for myelinated nerve cells, thyroid, gastric parietal cells, adrenals, smooth muscle, and mitochondria was tested using indirect immunofluorescent staining of appropriate human, rat, and monkey tissue sections.

Immunological memory—For evaluating the immunological memory, patients and controls were bled and given on day 0 0·2 ml of a standardised polyvalent vaccine containing 1/10 of the vaccinating dose of influenza A and B and tetanus and diphtheria toxoids. Patients and controls were bled again on day 21. A twofold dilution increase in antibody titres between days 0 and 21 was considered a positive reaction.

Measurement of circulating Blymphocytes—A modification of the technique of Jondal *et al*[12] was used to measure the number of B cells in peripheral blood. Smears of peripheral lymphocytes from the Ficoll-Hypaque technique were fixed in acetone at 20°C for 20 minutes. The smears were then washed with phosphate-buffered saline (PBS) for 5 minutes under constant agitation and incubated for 15 minutes with fresh human serum diluted 1/10 as a source of complement. The cells were then washed with PBS and incubated for 15 minutes with a FITC-conjugated goat antiserum to human C3. After three washings with PBS the lymphocytes were mounted in 1% buffered glycerin, covered with a coverslip, and read blindly under a Reichert microscope equipped with an Osramhbo-200 Mercury vapour lamp. The percentage of fluorescent lymphocytes was established on a total count of 100 lymphocytes.

Measurement of circulating T lymphocytes—The percentage of T lymphocytes was measured by the spontaneous T-rosette-forming cell technique described by Wybran *et al*.[13] In summary, 1×10^6 lymphocytes suspended in 1 ml of medium 199 were incubated with 20×10^6 sheep red blood cells (SRBC) in heat-inactivated and SRBC-absorbed fetal calf serum for one hour at 37°C. The percentage of lymphocytes that formed rosettes was calculated on 100 cells. A rosette-forming lymphocyte was called positive when three or more red cells adhered to that lymphocyte.

Lymphocyte transformation—The peripheral blood leucocytes from each patient were separated by the Ficoll-Hypaque technique. Then 1×10^6 lymphocytes/ml of medium 199, to which 10% fetal calf serum was added, were stimulated with phytohaemagglutin (PHA), concanavallin A, and pokeweed (PW) mitogens, as described.[14] To assess lymphocytes sensitised against central nervous tissue encephalitogens, blastic transformation and macrophage migration inhibition techniques were used.[14] Both techniques were performed with various concentrations of a human encephalitogenic protein prepared in our laboratory, a synthetic encephalitogenic peptide (11 amino-acids) synthesised and tested in our laboratory,[14] and the Beckman non-encephalitogenic peptide (Beckman, USA).

Production of the macrophage migration inhibition factor (MIF)—The MIF was produced by incubating 1×10^7 lymphocytes from each patient in 1 ml of medium 199 incorporating 5% of fetal calf serum for 24 hours at 37°C with the encephalitogenic protein, as reported.[15] Dilutions of the supernatants of 1/2, 1/4, and 1/10 were tested for MIF using capillary tubes filled with guinea-pig peritoneal exudate macrophages. Migration less than 75% after 24 hours was considered MIF-positive.

Delayed hypersensitivity skin tests—The delayed hypersensitivity status of the patients was assessed by intradermal injections of 0·1 ml of each of the following antigens: PPD at a concentration of 5 tuberculin units/ml (Connaught Medical Research Laboratories, Toronto, Canada); *Candida albicans* allergenic extract 1/100 (Hollister Stier Laboratory); mumps skin test antigens (Lilly); and streptokinase-streptodornase "varidase" (Lederle) at a concentration of 200 units streptokinase/ml and 50 units streptodornase/ml. The injection sites were examined after 48 hours and the induration and erythema measured in millimetres.

HLA tissue typing—The standard National Institute of Health (NIH) microlymphocytotoxicity test was used for tissue typing the patients with MS. Anti-HLA-3 (Bill Cannady; Denning; Galardo) HLA-7 (Moore; Stokes), and W 18 (Martin) were kindly supplied by the NIH.

Results

The duration of symptoms in the 12 women and 11 men with MS varied between 1 and 16 years. Sixteen of the 23 were ambulatory patients. Most of them had had more than four periods of exacerbation and remission (mean of 6·3), and all had had, on previous analyses,[9] a CSF IgG level over 0·035 g/l. Table I gives details of the patients' and controls' immunological status. Twice as many patients with MS as controls had had tonsillectomy and appendicectomy, and 18% of the patients compared with 4·5% of the controls had experienced repeated childhood infections. Though none of these factors separately are significant together, problems with tonsils and appendix and repeated infections may indicate inadequate immunological function.

HUMORAL IMMUNOLOGICAL FUNCTION

B lymphocytes—The relative percentage of circulating B lymphocytes in patients with MS was not different from that of the controls. The blastogenic transformation of circulating lymphocytes with PW mitogens and the level of serum immunoglobulins G, A, M, D, and E were similar in both groups.

Specific antibodies—There was no significant difference between the patients and controls in serum antibody titres against influenza A and B, parainfluenza I, II, and III, herpes simplex, and cytomegalovirus. But surprising and significant differences were found at day 0 between the two groups in their serum antibody titres against other viruses as well as for tetanus and diphtheria toxoids. A significantly higher percentage of patients with MS had a negative or weaker antibody titre than controls to adenovirus, mumps virus, and tetanus and diphtheria toxoids (table II). No patient with MS, for instance, had an antitetanus titre exceeding 1/512, while 48% of the controls exceeded this value ($P<0.0005$). The four distribution patterns strongly suggested the existence of many specific immunological deficiencies in the patients with MS. In contrast, higher serum measles and varicella zoster antibody titres were found in MS patients (table III). Forty per cent of MS patients compared with 13% of controls had a serum measles antibody titre over 1/32. Similarly none of the controls had a serum anti-varicella-zoster titre over 1/16 compared with 35% in the MS group.

Humoral mediated immunological memory—A similar percentage of patients and controls had a twofold increase in antibody titres against influenza A and B when patients were assessed 21 days after challenge

TABLE I—*Percentage of patients with MS and controls with personal (P) and family (F) histories of tonsillectomy, appendectomy, allergy, childhood infections, and cancer*

	Tonsillectomy	Appendicectomy	Allergy		Repeated infections in childhood		History of cancer	
			P	F	P	F	P	F
MS Patients	70	39	22	30	18	14	0	3·9
Controls	39	18	30	41	4·5	0	4·5	3·4
χ^2 Value on proportions (corrected)	0·10>P>0·05	0·4>P>0·2	P>0·50	P>0·50	0·4>P>0·2	0·4>P>0·2		P>0·50

TABLE II—*Percentage distribution of serum antibody titres against adenovirus, mumps virus, and diphtheria and tetanus toxoids in patients with MS and controls*

Titres:	0	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	P Value
						Adenovirus							
MS Patients	75	20	5										0·05<P<0·1
Controls	52	13	35										
						Viral mumps							
MS Patients	40	15	45	0									0·01<P<0·025
Controls	4	43	44	9									
						Diphtheria							
MS Patients				5	50	25	15	5	0	0			P<0·001
Controls				0	0	13	18	52	4	13			
						Tetanus							
MS Patients				5	11	21	37	26	0	0	0	0	0·025<P<0·05
Controls				0	4	4	9	35	22	4	18	4	

TABLE III—*Percentage distribution of serum antibody titres against measles and varicella zoster in patients with MS and controls*

Titres:	0	1/8	1/16	1/32	1/64	P Values
			Measles			
MS Patients	20	5	35	25	15	0·1<P<0·25
Controls	22	26	39	9	4	
			Varicella zoster			
MS Patients	45	20	30	5		0·01<P<0·025
Controls	74	26	0	0		

TABLE V—*HLA in patients with MS*

	No (%) of patients with each antigen	Significance (Student's *t* test)	No (%) of Caucasian kidney recipients from North America with each antigen
HLA-3	9/20 (45)	0·001<P<0·005	160/637 (25)
HLA-7	2/20 (10)	0·005<P<0·01	159/637 (25)
HLA, W 18	8/20 (40)	P<0·0001	83/637 (13)
HLA-3, HLA-7	0/23 (0)	0·01<P<0·025	50/637 (7·85)
HLA-3, W 18	7/23 (30)	P<0·0001	10/637 (1·57)
HLA-7, W 18	0/23 (0)	NS	6/637 (0·94)

NS = Not significant.

with the antigens. There was no statistical difference between the two groups and the distribution curves according to titres were similar. When the immune response was normal against one antigen the memory response also seemed to be normal. When the subjects were reassessed 21 days after the challenge, however, more of the controls had a twofold increase in antibody titres against diphtheria and tetanus toxoids (57% and 61% respectively) than the patients (35% and 42% respectively). This indicated that, like the primary response, the immunological memory function in patients with MS was also specifically impaired to some naturally occurring antigens.

Autoantibodies—Autoantibodies to thyroid, mitochondria, gastric parietal cells, smooth muscle, and myelinated nervous tissue were not detectable by the immunofluorescence test. ANF was found in six of the 23 controls (25%) and in 11 of the 23 patients (47%). An ANF titre greater than 1/10 was found in four patients but in none of the controls.

CELL-MEDIATED IMMUNOLOGICAL FUNCTION

T lymphocytes—No significant difference was found between patients with MS and controls in the percentage of T lymphocytes present. The mean ratios of transformation of the circulating lymphocytes with PHA and concanavallin A were also similar between the two groups.

Specific function of T lymphocytes—The specific function of the T lymphocytes was measured in vivo by their capacity to express a delayed type hypersensitivity reaction to four skin test antigens (table IV). The mean PPD reaction was twice as large in patients with MS as in the controls and there were more positive reactions among patients with MS. The mean delayed hypersensitivity reactions to mumps antigens and varidase were also higher in patients than in controls, but nearly three times as many controls as patients had a positive reaction to mumps.

Blastic transformation and MIF production—No significant difference between the controls and the patients in lymphocyte transformation or capacity to produce MIF was found with any of the three encephalitogens used. The dose-response effects also failed to show a difference. In some of the controls the lymphocytes were stimulated with the encephalitogens.

HLA in MS patients—HLA-3, HLA-7, and W 18 were typed in both MS and control lymphocytes (table V). Both HLA-3 and W 18 were found more often in patients with MS, as already reported,[16–19] than in the control group, and the difference was highly significant. The HLA-7 distribution was also different from that of the control group; HLA-7 was found in fewer patients with MS. The HLA-3 and W 18 combination was found in seven of the 23 MS patients compared with 10 out of 637 kidney recipients tested.

Discussion

We have shown that patients with MS have an increased incidence of appendicectomy, tonsillectomy, and childhood infections and significantly lower serum antibody titres against a variety of naturally occurring antigens. A low serum antibody titre against one antigen may often accompany higher antibody titres against others. These specific immune deficiencies seem to affect both the T- and B-cell systems as well as the immunological memory.

This increased susceptibility to infections and reduced capacity to mount an adequate immunological response to some bacterial and viral antigens reinforces the idea that there is a non-selective infection in these patients. Currier *et al*[20] observed a significant increase of infections before the age of 20 in patients with MS, and Panelius *et al*[21] found that patients with MS were more susceptible to childhood infections than their siblings. All these findings suggest that these multi-specific immunological abnormalities are primary rather than secondary to the disease activity and that patients with MS are partial or non-responders to many antigens. Even though these patients may be abnormal responders to many antigens they may be higher responders to other antigens—namely, measles and varicella zoster. Accumulating evidence favours this hypothesis. The higher antibody

TABLE IV—*Delayed hypersensitivity reactions to 4 antigens in patients with MS and controls*

	PPD		*Candida albicans*		Mumps		Varidase	
	Mean diameter (mm)	No with positive reaction	Mean diameter (mm)	No with positive reaction	Mean diameter (mm)	No with positive reaction	Mean diameter (mm)	No with positive reaction
MS Patients	15·9	15/23	8·1	12/23	15·2	7/23	28·1	21/23
Controls	7·4	12/23	8·1	21/23	6·8	19/23	22·4	22/23

titres against measles in patients with MS, as well as the delay in developing measles during childhood in these patients,[21] seems to indicate a high resistance against that virus. Panelius *et al*[21] showed not only that patients with MS contracted measles later than their siblings but also that men with MS tended to contract measles at an earlier age than women with the disease. The resistance of patients with MS to other types of micro-organisms has also been reported and supports the idea that such patients are higher responders to these micro-organisms.

If this hypothesis is true, serological studies might confirm the other observation of Panelius *et al* that histories of rheumatic fever were slightly less common in patients than in their siblings. This suggests that patients with MS have a higher resistance towards these micro-organisms. Nevertheless, nothing rules out the possibility that measles virus is directly related to the cause of the disease. The observation of Zabriskie *et al*[22] that there is a decreased cell-mediated response to paramyxovirus in patients with MS conflicts with this view, and this finding should perhaps be regarded as being similar to the abnormal PPD reaction found in patients with MS a few years ago.[3-5] These findings suggest that research, rather than focusing on a few exciting viruses or slow virus infections, should examine more closely the specific immune mechanism of patients with MS and its reaction to many types of infection.

The control of the immune response by genes related to the expression of the HLA antigens may be relevant. It has been well documented in animals that the genes controlling the expression of the transplantation antigens are closely linked to those controlling the expression of the immune response. Patients with MS have a higher incidence of HLA-3 and W 18 antigens than the normal population.[17-19] The association of HLA-3 and W 18 in 30% of our patients compared with only 1·5% of 637 kidney recipients is another interesting observation, and we are currently investigating the relation between this association and the evolution of the disease. Patients with MS have a mixed lymphocyte culture determinant in common, identical with that associated with the HLA-7 and W 18 in normal people.[23] All these results reinforce the increasing evidence that the locus similar in the mouse to the well-known lactate dehydrogenase (LD) locus that controls the expression of the immune response, abnormally controls this response in patients with MS.

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References

1 Kabat, E A, Moore, D H, and Landow, H, *Journal of Clinical Investigation*, 1942, **21**, 571.
2 McAlpine, D, Lumsden, C E, and Acheson, E D, *Multiple Sclerosis*, 2nd edn. Edinburgh and London, Churchill Livingstone, 1972.
3 Smith, H V, Hughes, I E, and Hunter, G, *Journal of Neurology, Neurosurgery and Psychiatry*, 1961, **24**, 101.
4 Marshall, J, and O'Grady, F, *Journal of Neurology, Neurosurgery and Psychiatry*, 1959, **22**, 277.
5 Miller, H, *et al*, *Journal of Neurology, Neurosurgery and Psychiatry*, 1961, **24**, 118.
6 Lamoureux, G, and Borduas, A G, *Clinical and Experimental Immunology*, 1966, **1**, 363.
7 Adams, J M, and Imagawa, D T, *Proceedings of the Society for Experimental Biology and Medicine*, 1962, **111**, 562.
8 Lamoureux, G, *et al*. In preparation.
9 Lamoureux, G, *et al*, *Neurology*, 1975, **25**, 537.
10 World Health Organisation, Respiratory Virus Disease, Technical Report Series No 170, p 48. Geneva, WHO, 1959.
11 Lamoureux, G, *et al*, *Annals de l'Institut Pasteur*, 1970, **118**, 562.
12 Jondal, M, Holm, G, and Wigzell, H, *Journal of Experimental Medicine*, 1972, **136**, 207.
13 Wybran, J, and Fudenberg, H, *Journal of Clinical Investigation*, 1973, **52**, 1026.
14 Lamoureux, G, *et al*, *Union Medicale du Canada*, 1972, **101**, 674.
15 Lamoureux, G, *et al*, *La Vie Médicale au Canada Français*, 1974, **3**, 479.
16 Jersild, C, Svejgaard, A, and Fog, T, *Lancet*, 1972, **1**, 1240.
17 Jersild, C, *et al*, *Tissue Antigens*, 1973, **3**, 243.
18 Naito, S, *et al*, *Tissue Antigens*, 1972, **2**, 1.
19 Bertrams, J, Kuwert, E, and Liedtke, U, *Tissue Antigens*, 1972, **2**, 405.
20 Currier, R D, *et al*, *Neurology*, 1974, **24**, 748.
21 Panelius, M, *et al*, *Acta Neurologica Scandinavica*, 1973, **42**, 89.
22 Utermohlen, V, and Zabriskie, J B, *Journal of Experimental Medicine*, 1973, **138**, 1591.
23 Jersild, C, *et al*, *Transplantation Proceedings*, 1973, **5**, 1791.

Intestinal absorption in normal Indian and English people

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Summary

The absorption of glycine, glycylglycine, water, and electrolytes was studied by intestinal perfusion in normal Indian and English people. Compared with the English people the Indians showed impaired absorption of all four substances. In the Indians the absorption of glycine and glycylglycine was impaired to the same extent, so that the kinetic advantage of glycylglycine as compared with glycine was preserved. The reduced absorption in the Indians may be the functional counterpart of the minor morphological changes seen in the jejunal mucosa of people living in southern India.

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Introduction

Normal Indian and English people differ in the structural appearance of their small intestinal mucosa.[1 2] Evidence of a functional difference, however, rests principally on studies of xylose and fat excretion,[3 4] which are at best indirect methods of quantifying absorption. We have therefore used the more direct method of intestinal perfusion to quantify and compare amino-acid and dipeptide absorption in apparently normal Indian and English people.

Subjects and methods

The English people (six men) had been studied previously in London by MDH.[5] The Indians (10 men) were admitted to this unit