PAPERS

Autoimmune T cell repertoire in optic neuritis and multiple sclerosis: T cells recognising multiple myelin proteins are accumulated in cerebrospinal fluid

Mats Söderström, Hans Link, Jia-Bin Sun, Sten Fredrikson, Zeng-Yu Wang, Weng-Xin Huang

Abstract

Monosymptomatic unilateral optic neuritis is a common first manifestation of multiple sclerosis. Abnormal T cell responses to myelin components including myelin basic protein (MBP), proteolipid protein (PLP), and myelin-associated glycoprotein (MAG) have been implicated in the pathogenesis of multiple sclerosis. Antigen-reactive T helper type 1 (Th1)-like cells that responded by interferon γ (IFN- γ) secretion on antigen stimulation in vitro were counted. Untreated patients with optic neuritis and multiple sclerosis had similarly raised levels of T cells recognising MBP, PLP, and MAG in peripheral blood. Such T cells were strongly enriched in CSF. None of these myelin antigens functioned as immunodominant T cell antigen characteristic for optic neuritis or multiple sclerosis. The autoimmune T cell repertoire was not more restricted in optic neuritis (as an example of early multiple sclerosis). The autoreactive T cell repertoires differed in blood compared with CSF in individual patients with optic neuritis and multiple sclerosis. No relations were found between specificity or quantity of auto-reactive T cells in blood or CSF, and clinical variables of optic neuritis or multiple sclerosis, or occurrence of oligoclonal IgG bands in CSF. The role of raised MBP, PLP, and MAG reactive Th1-like cells found in optic neuritis and multiple sclerosis remains unexplained.

(*J Neurol Neurosurg Psychiatry* 1994;57:544-551)

Multiple sclerosis is characterised histopathologically by patchy demyelination as well as perivascular accumulations of mononuclear cells in the form of macrophages, T cells, and B cells. Another characteristic feature of multiple sclerosis is the evidence of abnormal B cell activation, as reflected in the cerebrospinal fluid (CSF) by raised immunoglobulin concentrations and the presence of oligoclonal IgG bands.¹ Several specific antibodies originating from the CNS and the CSF have been described in multiple sclerosis,

including those directed against different viruses and the myelin constituents myelin basic protein (MBP), proteolipid protein (PLP), myelin associated glycoprotein (MAG), and myelin oligodendrocyte glycoprotein (MOG).²⁻⁷ These B cell responses comprise mainly the IgG isotype and can therefore be assumed to depend on T cell help. The finding that experimental allergic encephalomyelitis—which in its chronic form mimics multiple sclerosis both clinically and pathologically—is characterised by high concentrations of MBP reactive T cells and can be transferred with MBP specific T cells to naive recipients,⁸ has led to renewed efforts to investigate T cell specificities to myelin proteins in patients with multiple sclerosis.

The traditional ways to assess T cell sensitisation include measurements of ³H-thymidine incorporation as a reflection of antigen-induced T cell proliferation, and limiting dilution assays. Such studies have given variable results, although most of them concluded that a low percentage of patients with multiple sclerosis showed evidence of T cell sensitisation to MBP and PLP, and to MBP and PLP peptides, as did a somewhat lower percentage of control subjects.⁹ These techniques may, however, not be sensitive enough to detect the low frequencies of memory T cells that occur in human diseases with possible autoimmune background, nor are they useful to detect antigen reactive T cells among the low numbers of CSF cells usually available. A different approach that is based on counting cells that secrete an effector molecule in the form of interferon γ (IFN- γ) after in vitro stimulation with antigen, allows the definition of the T cell repertoire for specificity, quantity, and functional differentiation as far as the CD4⁺ T helper cell subtype 1 (Th1) related effector molecule IFN- γ is concerned. After phagocytosis of myelin protein antigen by macrophages and processing, sequences comprising 10–20 amino acids join congruent major histocompatibility complex (MHC) class II molecules and are presented on the cell surface together with these molecules to T cells which recognise the antigen-MHC unit via the T cell receptor, thereby inducing the production and secretion of effector molecules including IFN- γ .¹⁰ Patients with multiple sclerosis had

Karolinska Institutet,
Huddinge Hospital,
Stockholm, Sweden
Department of
Neurology

H Link
J-B Sun
S Fredrikson
Z-Y Wang
W-X Huang

Department of
Ophthalmology
M Söderström

Correspondence to:
Dr Mats Söderström,
Department of
Ophthalmology, Karolinska
Institutet, Huddinge
Hospital, S-141 86
Huddinge, Sweden.

Received 5 April 1993
and in revised form
9 August 1993.
Accepted 17 August 1993

increased concentrations of Th1-like cells secreting IFN- γ on recognition of MBP, PLP, MAG, and MOG, and different MBP peptides, without immunodominance for any of the myelin protein antigens or peptides hitherto examined.^{6,11–14} When applied to CSF cells, it was shown that patients with multiple sclerosis had a strong accumulation of T cells with these autoreactivities in the CSF compartment.

Whether T cells in individual patients with multiple sclerosis respond to more than one myelin protein antigen in parallel has not been studied. Furthermore, it is not known whether a more restricted autoimmune T cell response occurs in early compared to late phases of multiple sclerosis. One rather common clinical manifestation that often represents the first clinical sign of multiple sclerosis is monosymptomatic unilateral optic neuritis. About half of the patients with a first attack of optic neuritis later develop multiple sclerosis. Here we report the results from studies of the T cell responses to MBP, PLP, and MAG in blood and CSF from patients with multiple sclerosis, optic neuritis, and other neurological diseases.

Patients and methods

PATIENTS

Optic neuritis

Thirty-two patients (26 women) were included. Their mean age was 36 (18–53) years. The interval between first symptoms of optic neuritis and present study was five days to three years (median 34 days). To be included, the patients must not have had any previous history of neurological disturbances that could in any way be related to multiple sclerosis, although recurrent optic neuritis in four patients was accepted. A complete ophthalmological and neurological examination including vibrometry for evaluation of deep sensation was performed in all patients and was normal except for findings related to optic neuritis. Examinations for visual evoked potentials, x-ray film studies, and CT of the brain and the orbit were performed on selected patients based on clinical judgement. All patients were referred for MRI. Nine refused to participate and four could not be examined because of pregnancy (one), adipositas (one), and claustrophobia (two). Thirteen of the 19 patients (68%) had three or more high signal lesions on brain MRI. The interval between first symptoms of optic neuritis and MRI was 19 days to three years (median 120 days), and between CSF sampling and MRI nine to 180 days (median 86 days). During the interval between sampling and MRI, none of the patients reported worsening of optic neuritis or any new neurological signs or symptoms. Thirty-one patients with optic neuritis underwent lumbar puncture. Twenty (65%) had mononuclear pleocytosis ($>5 \times 10^6/l$), three (10%) had a slight increase in the CSF to plasma albumin ratio reflecting damage to the blood-CSF barrier, 13 (42%) had an increased (>0.7) CSF IgG index, and 25 (81%) had oligoclonal IgG bands demonstrated by agarose

isoelectric focusing combined with immunoblotting and avidin-biotin amplified double-antibody peroxidase staining.¹⁵ All 13 patients MRI positive for multiple sclerosis had oligoclonal bands in their CSF. None of the patients with optic neuritis had been treated with immunomodulatory drugs except for one patient with optic neuritis two years previously, when she received corticosteroids.

Multiple sclerosis

Thirty-nine patients (28 women) had clinically definite multiple sclerosis.¹⁶ Their mean age was 38 (range 20–53) years. The duration of multiple sclerosis was 1–32 years (mean 7.6 years). The patients were divided into three groups regarding disease progression: (1) exacerbation—there had been a sudden appearance of new symptoms and signs or a sudden reappearance or worsening of previous findings lasting more than 24 hours within four weeks before examination; (2) remission; or (3) chronic progressive phase of multiple sclerosis. Twelve, 18, and nine patients, respectively, belonged to these three groups. Twenty-six of the patients had no or only slight disability—that is, they were in all respects able to manage on their own—and 13 patients had moderate or severe disability, implying dependence on assistance to various degrees in the patient's professional and social life. A "malignancy" index equal to the ratio of disability to duration in years was calculated. No disability was assigned a value of 1, slight disability 2, moderate disability 3, and severe disability 4. The median value of the malignancy index in the 39 patients with multiple sclerosis was 0.36. The patients were separated into two groups, one with index values <0.36 and a more benign course (19 patients), and one with index values >0.36 and a less benign course (20 patients). CSF was obtained from all patients with multiple sclerosis and revealed mononuclear pleocytosis in 25 (64%), slightly increased CSF to plasma albumin ratio in seven (18%), a raised IgG index in 30 (77%), and oligoclonal IgG bands in all 39. No patient with multiple sclerosis had ever been treated with immunomodulatory drugs.

Other neurological diseases

Fifty-eight patients (33 women) belonged to this group. Their mean age was 45 (range 16–81) years. The diagnosis was acute aseptic meningoencephalitis in 31 patients, muscular tension headache in seven, peripheral neuropathy in six, cerebrovascular disease in four, epilepsy in three, and Lyme neuroborreliosis, Bell's palsy, migraine, cerebral vasculitis, vertigo, dementia, and paraesthesia in one patient each. Studies of CSF revealed mononuclear pleocytosis in 32 patients (55%), a slightly increased CSF to plasma albumin ratio in 16 (28%), a raised IgG index in six (10%), and oligoclonal IgG bands in CSF in only 10 (17%).

The study protocol was approved by the ethics committee of Karolinska Institutet at Huddinge Hospital and informed consent was obtained from all patients.

ANTIGENS

MBP was prepared from human white matter.¹⁷ Purity of MBP was checked by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), which showed a single band migrating at about 22 kDa. PLP was isolated from bovine brain.¹⁸ Purity was assessed by SDS-PAGE. It was free from any contamination with MBP as checked by western blotting. MAG was prepared by the lithium diiodosalicylate phenol method¹⁹ from bovine brain myelin. Purity was checked by SDS-PAGE which revealed a single band migrating at 100 kDa, and by western blotting using serum from a patient with monoclonal anti-MAG IgM which bound to the single 100 kDa band.

PREPARATION OF MONONUCLEAR CELLS FROM CSF AND BLOOD

A 20-ml volume of CSF was collected into siliconised glass tubes and centrifuged for 10 minutes at 200 *g*. The cells in the pellet were resuspended in Iscove's modification of Dulbecco's medium supplemented with 2 mM L-glutamine, 1% (v/v) minimal essential medium (all from Flow laboratories, Irvine, UK), 10% (v/v) fetal calf serum (Gibco, Paisley, UK), and antibiotics, centrifuged and rediluted to $4-10 \times 10^4$ cells/ml. Venous blood was collected into heparinised glass tubes and diluted with the same volume of medium. Mononuclear cells were obtained by density gradient centrifugation on Lymphoprep (Nyegaard, Oslo, Norway). The cells from the interphase were carefully collected and washed three times with medium and antibiotics, resuspended in medium, counted, and adjusted to 10^6 cells/ml.

ENUMERATION OF MYELIN PROTEIN REACTIVE T CELLS

Antigen specific T cells in CSF and blood were counted in an IFN- γ immunospot assay as previously described.¹¹ Briefly, 200 μ l aliquots containing 2×10^5 blood mononuclear cells in duplicate or 100 μ l aliquots containing $4-10 \times 10^5$ CSF cells (rarely in duplicate because of low numbers of CSF cells available) were added to individual wells of microtitre plates with 96 wells and nitrocellulose bottoms, which were coated with a mouse monoclonal anti-human IFN- γ antibody (7-B6-5).²⁰ The antigens (MBP, PLP, or MAG) at a concentration of 200 μ g/ml were added in volumes of 10 μ l/well. This antigen concentration gave maximum numbers of spots in preliminary experiments. For each specimen, wells receiving no antigen were used as background control cultures (0 antigen), and wells receiving purified protein derivative (PPD; Statens Seruminstitut, Copenhagen, Denmark) or phytohaemagglutinin (PHA; Difco, Detroit, MI, USA) at final concentrations of 10 and 1 μ g/ml, respectively, were used as positive controls. After incubation of cultures for 48 hours and washing, IFN- γ captured at the site of its secretion was detected by adding a rabbit polyclonal anti-human IFN- γ antiserum (Interferon Sciences, New Brunswick, NJ, USA). The

plates were incubated and washed, and biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA) was added, followed by avidin-biotin peroxidase complex (ABC Vectastain Elite Kit; Vector) and peroxidase staining. The red-brown spots that corresponded to the cells that had secreted IFN- γ were counted with a dissection microscope under $\times 25$ magnification. No spots appeared when the rabbit polyclonal anti-human IFN- γ antiserum was substituted by a mouse anti-rat IFN- γ antibody or an irrelevant mouse monoclonal antibody, or when the rabbit polyclonal antibody was omitted. Results were expressed as numbers of spots/ 10^5 mononuclear cells after subtraction of values in the background control cultures with no antigen added. Because in some patients the numbers of IFN- γ secreting cells were higher in the control cultures than in cultures exposed to antigen, subtraction in these instances resulted in negative values. A patient was considered a responder to a given antigen if the value after subtraction was above 0. All countings were performed without knowledge of the clinical diagnosis or the antigen employed. The variation of quadruplicate cultures in preliminary experiments was regularly less than 10%. The spots detected in the assay are the results of MHC restricted antigen presentation to T cells and reflect individual antigen reactive T cells secreting IFN- γ .^{13 21}

STATISTICS

The Mann-Whitney test was used.

Results

MYELIN PROTEIN REACTIVE T CELLS IN BLOOD AND CSF IN OPTIC NEURITIS

Most of the patients with optic neuritis had MBP, PLP, and MAG reactive T cells in peripheral blood (fig 1; table 1). The levels of such cells were similar in optic neuritis and multiple sclerosis. The median number of MBP reactive T cells in the patients with optic neuritis was $1.3/10^5$ blood mononuclear cells, corresponding to roughly 1/77 000 cells, and in multiple sclerosis $1/10^5$ cells. The median number of PLP reactive T cells in optic neuritis was $0.8/10^5$ blood cells (1 per 125 000 cells), and in multiple sclerosis 2 per 10^5 (1/50 000 cells), and the median number of MAG reactive T cells in optic neuritis was $0.3/10^5$ mononuclear cells (1/330 000 cells), and in multiple sclerosis $1/10^5$ cells. Patients with optic neuritis or multiple sclerosis had higher numbers of T cells with these reactivities in blood compared with the patients with other neurological diseases.

In CSF, MBP, PLP, and MAG reactive T cells were detected in most of the patients with optic neuritis. As in blood, no significant differences were found between optic neuritis and multiple sclerosis (table 1; figs 1 and 2). The median value of MBP reactive T cells in optic neuritis was 55 and in multiple sclerosis $50/10^5$ CSF cells, corresponding to roughly 1/1800 and 1/2000 cells, respectively; of PLP reactive T cells in optic neuritis was 50 and

Figure 1 Numbers of cells secreting IFN-γ/10⁵ mononuclear cells cultivated in the absence of antigen (0) and in the presence of MBP, PLP, and MAG, in patients with optic neuritis (ON), multiple sclerosis (MS), and other neurological diseases (OND). (A) Data for blood; (B) CSF.

Table 1 Numbers of cells secreting IFN-γ/10⁵ mononuclear cells from peripheral blood and CSF from patients with optic neuritis (ON), multiple sclerosis (MS), and other neurological diseases (OND) after stimulation with the myelin proteins MBP, PLP, MAG, the recall antigen PPD, and PHA

Diagnostic group	Blood						CSF			
	MBP	PLP	MAG	PPD	PHA	0 Ag	MBP	PLP	MAG	0 Ag
ON:										
Range	-2 to 17	-4.5 to 19.5	-6 to 5	1.5 to 198	16 to 398	0 to 6	-60 to 1090	0 to 800	0 to 470	0 to 1200
Mean (SD)	2.4 (4.1)	1.6 (4.2)	0.4 (2.2)	22.9 (35.9)	122.7 (92.2)	1.9 (1.8)	117 (213)	165 (236)	199 (212)	195 (280)
Median	1.3	0.8	0.3	9.8	99	1.5	55	50	120	70
MS:										
Range	-7 to 60	-5 to 138	-7.5 to 42	2 to 160	3 to 400	0 to 27	-400 to 1080	0 to 1560	0 to 470	0 to 1900
Mean (SD)	6.3 (15.6)	9.0 (29)	2.4 (8.4)	27.3 (29.8)	159.4 (106.7)	8.8 (11.5)	69 (230)	258 (431)	159 (165)	341 (453)
Median	1.0	2.0	1.0	15	146	3.4	50	60	105	160
OND:										
Range	-11 to 25	-20 to 101	-81 to 6.5	2 to 183	7 to 396	0 to 90	-1000 to 850	-200 to 410	-300 to 480	0 to 2200
Mean (SD)	-0.4 (5.2)	2.7 (19.3)	-4.1 (15.1)	29.0 (32.1)	145.4 (95.2)	6.1 (11.9)	-56 (217)	27 (110)	16 (146)	232 (380)
Median	0	-1	-0.5	18	143.5	3.8	0	0	0	80
p Values:										
ON v MS	NS	NS	NS	NS	NS	< 0.05	NS	NS	NS	NS
ON v OND	0.01	0.05	0.05	NS	NS	0.05	0.001	0.05	0.01	NS
MS v OND	0.01	0.05	0.01	NS	NS	NS	0.01	0.001	0.01	NS

Data refer to values obtained after subtraction of values from background control cultures propagated in the absence of myelin protein (0 Ag). In some patients the numbers of cells secreting IFN-γ were higher in 0 Ag control cultures than in antigen stimulated cultures, explaining the negative values for antigen specific IFN-γ secreting cells registered in some instances; NS = not significant.

Figure 2 Numbers of (A) MBP, (B) PLP, and (C) MAG reactive T cells/ 10^5 mononuclear cells from CSF from patients with optic neuritis (ON), multiple sclerosis (MS), and other neurological diseases (OND). Data presented refer to values obtained after subtraction of values from background control cultures propagated without antigen. Negative values (compare table 1) are arbitrarily presented as 0.

in MS $60/10^5$ CSF cells, corresponding to $1/2000$ and $1/1700$ cells, respectively; and of MAG reactive T cells in optic neuritis was 120 and in MS $105/10^5$ CSF cells corresponding to about $1/800$ and $1/1000$ CSF cells, respectively. The numbers in optic neuritis and multiple sclerosis were significantly higher when compared with other neurological diseases (table 1). Throughout, the numbers of myelin protein reactive T cells were higher in CSF compared with blood. This enrichment in CSF was 42-fold for MBP reactive T cells in optic neuritis and 50-fold in multiple sclerosis, and for PLP reactive T cells 63-fold in optic neuritis and 30-fold in multiple sclerosis. A similar enrichment in CSF was found for MAG responsive T cells in both optic neuritis and multiple sclerosis. In some of the patients with other neurological diseases, an accumulation of myelin protein responsive T cells in CSF was also found. The diagnoses in these patients included both inflammatory and non-inflammatory neurological diseases. For example, the patient with neuroborreliosis had 0 and 50 MBP, and 10 and 100 PLP responding T cells/ 10^5 mononuclear cells in blood and CSF, respectively; the patient with dementia had 0 and 250 MBP responding T cells; the patient with cerebral vasculitis had 1 and 170 MBP, and 0 and 170 PLP responding T cells; a patient with epilepsy had 0 and 100 MBP, and another patient with epilepsy had 0 and 40 MAG responding T cells; and the patient with Bell's palsy had 2 and 40 MBP, and 4 and 220 PLP responding T cells/ 10^5 mononuclear cells in blood and CSF, respectively.

No differences were found between the three patient groups for T cell responses in blood to the recall antigen PPD or to PHA (table 1), thereby underlining that the differences regarding T cell autoreactivities between optic neuritis, multiple sclerosis, and other neurological diseases are not due to a general, non-specific T cell hyperreactivity. MNC in CSF could not be examined for responses to PPD or PHA because of the low numbers of cells available.

Numbers of cells secreting IFN- γ in cultures with no antigen or PHA added were somewhat lower in blood from the patients with optic neuritis, whereas no differences were found in CSF between the three patient groups (table 1).

ARE MULTIPLE MYELIN PROTEINS RECOGNISED BY T CELLS FROM INDIVIDUAL PATIENTS?

Blood from 16 patients with optic neuritis, 17 with multiple sclerosis, and 13 with other neurological diseases was examined for T cell reactivities to all three myelin proteins in parallel. T cells responding to MBP + PLP + MAG were detected in two patients with optic neuritis (13%), whereas eight (50%) had T cells recognising two of the myelin antigens and three patients with optic neuritis (19%) had T cells responding to one of the antigens only (table 2). Three patients with optic neuritis (19%) had no T cells in blood responding to any of the myelin proteins. Of the patients with multiple sclerosis, seven (41%) showed T cell responses to MBP + PLP + MAG, four patients (24%) to only two myelin proteins (always MBP and PLP), and six (35%) to only one protein. None of the patients with multiple sclerosis lacked T cell responses to myelin proteins. Of the patients with other neurological diseases, three (23%) responded to MBP + PLP + MAG (the diagnosis was acute meningoencephalitis in two patients and polyneuropathy in one), whereas five patients (38%) had T cells responding to only one of the myelin proteins, and five patients (38%) did not respond.

Cells in CSF from a few patients were also examined for T cell responses to MBP, PLP, and MAG in parallel (table 2). Six of 10 patients with optic neuritis had T cells responding to MBP + PLP + MAG, two

Table 2 Numbers of patients with optic neuritis (ON), multiple sclerosis (MS), and other neurological diseases (OND) recognising different numbers of myelin protein antigens in the IFN- γ immunospot assay

Diagnostic group	No of myelin antigens recognised by T cells			
	3	2	1	0
ON:				
Blood (n = 16)	2 (13)	8 (50)	3 (19)	3 (19)
CSF (n = 10)	6 (60)	2 (20)	1 (10)	1 (10)
MS:				
Blood (n = 17)	7 (41)	4 (24)	6 (35)	0
CSF (n = 6)	2	1	3	0
OND:				
Blood (n = 13)	3 (23)	0	5 (38)	5 (38)
CSF (n = 6)	1	1	0	4

Numbers in parentheses are percentages.

patients had T cells recognising two proteins, and one patient had T cells responding to only one of the myelin protein antigens. One patient did not respond. Among six patients with multiple sclerosis similarly examined, two had T cells recognising MBP + PLP + MAG, one had T cells responding to PLP and MAG, and the remaining three patients had MAG reactive T cells only. Of six patients with other neurological diseases studied, one patient who had aseptic meningoencephalitis had T cells responding to MBP +

Table 3 Numbers of patients with optic neuritis (ON), multiple sclerosis (MS), and other neurological diseases (OND) with T cells from blood and CSF recognising predominantly MBP, PLP, or MAG

Diagnostic group	MBP	PLP	MAG	Identical response against two different myelin proteins	No response
ON:					
Blood (n = 16)	9	3	1	0	3
CSF (n = 10)	3	2	3	1	1
MS:					
Blood (n = 17)	6	3	7	1	0
CSF (n = 6)	0	2	4	0	0
OND:					
Blood (n = 13)	5	1	2	0	5
CSF (n = 6)	0	1	1	0	4

Table 4 Numbers of antigen-specific T cells secreting IFN- γ /10⁵ mononuclear cells from blood and CSF from individual patients with optic neuritis (ON), multiple sclerosis (MS), and other neurological diseases (OND) after stimulation with MBP, PLP, and MAG in parallel

Patient	Blood			CSF		
	MBP	PLP	MAG	MBP	PLP	MAG
ON:						
1	2	1.5	1	1090	0	20
2	2	0	0	140	540	240
3	0	4.5	0	120	120	320
4	0	19.5	0.5	0	0	400
5	0	0	0	40	180	140
6	1	0	0.5	50	100	120
7	4	0	1.5	140	0	80
8	0	0	0	80	30	50
9	0	1.5	0	40	20	40
10	0	0	0	0	0	0
MS:						
1	2	2.5	3.5	90	370	470
2	60	4	2	100	300	100
3	0.5	13.5	0	0	0	100
4	0.5	0	0	0	50	50
5	0	0	2	0	0	20
6	0	1	0	0	0	20
OND:						
1	3	2	0.5	0	140	480
2	0.5	0	0	40	410	160
3	0	0	1	0	0	0
4	0	0	0.5	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0

Data are values obtained after subtraction of values for control cultures propagated in absence of myelin antigen

PLP + MAG, one patient with inflammatory polyneuropathy had T cells recognising PLP and MAG, and the remaining four patients had no detectable T cell autoreactivities in CSF (patients Nos 1–6 in table 4).

IS MBP, PLP, OR MAG IMMUNODOMINANT IN OPTIC NEURITIS OR MULTIPLE SCLEROSIS?

Any of the three myelin proteins functioned as an immune dominant T cell antigen in individual patients with optic neuritis, multiple sclerosis, and other neurological diseases (table 3). The results did not disclose any multiple sclerosis or optic neuritis specific T cell antigen among MBP, PLP, and MAG.

To study the predominant T cell response in individual patients and compare mononuclear cells in blood *v* CSF of the same patient, T cell responses to MBP, PLP, and MAG were examined in parallel in blood and CSF from 10 patients with optic neuritis, six with multiple sclerosis and six with OND (table 4). One patient with optic neuritis and two with other neurological diseases had no responding T cells either in blood or in CSF. Only two of the 10 patients with optic neuritis (Nos 1 and 7) had a dominant T cell response to the same antigen (MBP) in both blood and CSF whereas, in the remaining seven patients with optic neuritis, the dominant T cell antigen varied in the individual patient when blood and CSF data were compared. Two patients with optic neuritis (Nos 5 and 8) had MBP reactive T cells in CSF only. Among the six patients with multiple sclerosis, synchronous immune dominance in blood and CSF to MAG was found in two patients (Nos 1 and 5). The remaining four patients with multiple sclerosis and the four with other neurological diseases showed different patterns of T cell responses to MBP, PLP, and MAG when results from the simultaneous studies on mononuclear cells in blood and CSF were compared. Thus the autonomy of the intrathecal T cell response was not only reflected by the much higher numbers of myelin protein reactive T cells in CSF compared with blood (table 1), or the presence of such cells in CSF only (table 4), but also by differences with regard to myelin protein antigen that was predominantly recognised by T cells when blood and CSF obtained from individual patients were compared (table 4).

RELATION BETWEEN MYELIN PROTEIN REACTIVE T CELLS, OLIGOCLONAL BANDS IN CSF, AND MRI FINDINGS IN PATIENTS WITH OPTIC NEURITIS

Thirteen of the 19 patients with optic neuritis examined by MRI had symptom free intracerebral lesions consistent with those seen in multiple sclerosis (see under PATIENTS). No differences in numbers of T cells recognising individual myelin proteins, or the sum of MBP, PLP, and MAG responding T cells in blood or CSF were seen in MRI positive compared with MRI negative patients with optic neuritis.

Oligoclonal IgG bands were detected in CSF from 25 of the 31 patients with optic

neuritis who were examined by lumbar puncture. No correlation was found between numbers of myelin protein antigen reactive T cells in blood or CSF and occurrence of oligoclonal bands, nor was there any relation to CSF cell counts. Also in those 10 patients with optic neuritis in whom T cell responses to MBP, PLP, and MAG were analysed in parallel, no relations were found between the sum of MBP, PLP, and MAG reactive T cells in CSF or blood and oligoclonal bands.

RELATION BETWEEN MYELIN PROTEIN REACTIVE T CELLS AND CLINICAL VARIABLES OF OPTIC NEURITIS AND MULTIPLE SCLEROSIS

The 32 patients with optic neuritis were divided into two groups of equal size with regard to short *v* long interval between onset of clinical signs and symptoms of optic neuritis and the time of CSF and blood sampling. Numbers of T cells responding to each of the three myelin protein antigens, or the sum of MBP + PLP + MAG reactive T cells, did not differ significantly between groups with short or long intervals.

The patients with multiple sclerosis were divided into those with clinical exacerbation *v* remission or chronic progressive multiple sclerosis, duration of disease <8 years compared with >8 years, no or slight *v* moderate or severe disability, and a more *v* less benign course based on the outcome of the malignancy index (see under PATIENTS). No statistically significant differences between numbers of T cells in blood or CSF responding to any of the three myelin proteins, or the sum of MBP + PLP + MAG reactive T cells could be found in any of these clinical subgroups. No correlation was found between the clinical variables studied and levels of myelin protein responding T cells.

Discussion

Patients with optic neuritis had autoimmune T cells in their CSF recognising the three major myelin proteins MBP, PLP, and MAG—that is, myelin components that could constitute targets for a putative autoimmune attack in multiple sclerosis. The proportions of these autoimmune T cells in CSF were similar in optic neuritis and multiple sclerosis, suggesting that there was no restriction of the T cell repertoire in early multiple sclerosis as reflected by optic neuritis. Also in peripheral blood, increases of T cells with these specificities were detected both in patients with optic neuritis and multiple sclerosis, but the relative numbers of T cells with these specificities were much lower than in CSF. In the search for a “multiple sclerosis antigen”, we have for the first time also defined the T cell repertoire in individual patients to different myelin proteins (MBP, PLP, and MAG) in parallel. No immunodominant response to any of these myelin protein antigens was found in either optic neuritis or multiple sclerosis. Instead, T cells from individual patients responded predominantly to one or the other of these myelin proteins. Different response patterns were found in CSF compared with

blood in individual patients as further evidence of an autonomous autoimmune T cell response in the CSF compartment.

It has previously been documented that the presently used IFN- γ immunospot assay is a useful tool to examine the T cell repertoire regarding specificity and quantity.^{11–14,21} IFN- γ has many functions, including induction of MHC class II antigens on targets of the autoaggressive attack in organ-specific autoimmune diseases—for example, pancreatic β cells in nude mice,²² thyroid epithelial cells in obese strain chickens,²³ and astrocytes in mouse and rat strains susceptible for the induction of experimental allergic encephalitis.²⁴ It has also been shown that IFN- γ , when given systemically, worsens multiple sclerosis.²⁵ The present study clearly showed that multiple sclerosis as well as optic neuritis is accompanied by a strong enrichment in the CSF of Th1-like cells secreting IFN- γ on recognition of MBP, PLP, and MAG. Raised concentrations of MBP occur regularly in CSF from patients with multiple sclerosis. The continuous process of IFN- γ secretion, as envisaged by the inflammatory lesions affecting the brain in multiple sclerosis, could have an important influence on the course of the disease.

We subtracted the numbers of IFN- γ secreting cells obtained in background control cultures with no antigen added from numbers in cultures exposed to antigen (MBP, PLP, or MAG). In some patients the numbers of IFN- γ spots were higher in the control cultures without antigen than in cultures exposed to antigen, thereby explaining the negative values for numbers of myelin antigen specific T cells sometimes found (table 1). This could be due to effects of immune regulation during culture, such as secretion of cytokines (IL-4, transforming growth factor β) downregulating cells secreting IFN- γ . The presence of IFN- γ spots in control cultures without antigen added (table 1) might represent T cell recognition of endogenous antigen carried by antigen presenting cells in the sample, T cell recognition of exogenous components in the culture medium, or T cells committed to secretion of IFN- γ already *in vivo*.

Antigen recognition by CD4⁺ Th cells most probably results not only in secretion of IFN- γ by antigen specific Th1-like cells, but also in IL-4 secretion via Th2 cells. These subgroups of Th cells have been most clearly defined in mice, but they probably also occur in humans.²⁶ The predominant involvement of Th1 or Th2 cells with accompanying differences in cytokine patterns might be important in the development and outcome of inflammatory responses.

A major question is whether myelin protein reactive T cells play a part in the pathogenesis of multiple sclerosis. Such cells have been detected not only in multiple sclerosis but also in other inflammatory neurological diseases, and even in diseases of the nervous system that are usually not associated with overt inflammation—for example, cerebrovascular diseases.²⁷ Also in the present study, patients

with aseptic meningoencephalitis, cerebrovascular diseases, polyneuropathy (data not shown), dementia, and epilepsy showed strong accumulation of myelin protein responsive T cells in CSF. The numbers of autoimmune T cells found in these diseases may reach the same levels both in blood and CSF as have been found in multiple sclerosis and optic neuritis. The persistence of high levels of myelin protein and peptide reactive T cells, as can be anticipated to take place in multiple sclerosis and optic neuritis might, however, influence the initiation and perpetuation of immune mediated demyelination via the release of effector substances including IFN- γ .

One impetus for the present study of patients with optic neuritis regarding T cell responses to putative myelin autoantigens of possible relevance in the immunopathogenesis of multiple sclerosis was to analyse whether a more restricted T cell repertoire occurred in optic neuritis compared with clinically definite multiple sclerosis. In experimental allergic encephalitis induced in Lewis rats, the development over time of T cells responding to different MBP peptides has been analysed with the IFN- γ immunospot assay. Interestingly, the firstly demonstrable T cell response, which occurred in the inductive phase of the immune response and before clinical signs and symptoms, was directed to the encephalitogenic peptide of the Lewis rat. During clinical experimental allergic encephalitis, multiple MBP peptides were recognised by T cells, and any immune dominance of the encephalitogenic peptide could no longer be found.²⁸ A spreading of T cell epitopes has also been reported in a chronic experimental allergic encephalitis model in mice.²⁹ On comparison of optic neuritis with multiple sclerosis, no differences regarding specificities or numbers of myelin protein responsive T cells were found in blood or CSF. The data indicate that optic neuritis, even when reflecting early multiple sclerosis, is characterised by a T cell repertoire of the same complexity regarding autoreactivities as clinically definite multiple sclerosis, and involving not only MBP, but PLP and MAG as well. The goal to detect more restricted T cell responses in early multiple sclerosis to establish a possible basis for individualised immune therapy, therefore remains unfilled.

We thank Dr M Lindqvist, Department of Neuroradiology, Karolinska Hospital, for examining the MRIs, and H-P Ekre, Department of Immunology, University of Lund, for a generous gift of the antibody 7-B6-5. This study was supported by grants from the Swedish MS Society (NHR), the Swedish Medical Research Council, and research funds of the Karolinska Institutet. Dr Jia-Bin Sun is a guest researcher from the Department of Neurology, First Hospital, Harbin Medical University, and Dr Weng-Xin Huang from the Department of Biochemistry, Sun Yat-Sen University of Medical Sciences, Guangzhou, Peoples' Republic of China.

- 1 Link H. The cerebrospinal fluid in multiple sclerosis. In: Swash M, Oxbury J, eds. *Textbook of neurology*. Edinburgh: Churchill Livingstone, 1991:1128-39.
- 2 Norrby E, Link H, Olsson JE, Panelius M, Salmi A, Vandvik B. Comparison of antibodies against different

- viruses in cerebrospinal fluid and serum samples from patients with multiple sclerosis. *Infect Immun* 1974;10: 688-94.
- 3 Wajgt A, Gorny M. CSF antibodies to myelin basic protein and to myelin associated glycoprotein in multiple sclerosis. Evidence of the intrathecal production of antibodies. *Acta Neurol Scand* 1983;68:337-43.
- 4 Möller JR, Johnson D, Brady RO, Tourtellotte WW, Quarles RH. Antibodies to myelin-associated glycoprotein (MAG) in the cerebrospinal fluid of multiple sclerosis patients. *J Neuroimmunol* 1989;22:55-61.
- 5 Olsson T, Baig S, Höjberg B, Link H. Antimyelin basic protein and antimyelin antibody producing cells in multiple sclerosis. *Ann Neurol* 1990;27:132-6.
- 6 Sun J, Olsson T, Wang WZ, et al. Autoreactive T and B cell responding to myelin proteolipid protein in multiple sclerosis and controls. *Eur J Immunol* 1990;21:1461-8.
- 7 Xiao BG, Linington C, Link H. Antibodies to myelin-oligodendrocyte glycoprotein in cerebrospinal fluid from patients with multiple sclerosis and controls. *J Neuroimmunol* 1991;31:91-6.
- 8 Ben-Nun A, Wekerle H, Cohen I. The rapid isolation of clonable antigen specific T-lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur J Immunol* 1981;11:195-9.
- 9 Sun JB. Autoreactive T and B cells in nervous system diseases. *Acta Neurol Scand* 1993;87(suppl 142):1-56.
- 10 Hecht TT, Longo DL, Matis A. The relationship between immune interferon production and proliferation in antigen-specific MHC-restricted T cell lines and clones. *J Immunol* 1983;131:1049-55.
- 11 Olsson T, Wang WZ, Höjberg B, Kostulas V, Jiang YP, Andersson G, et al. Autoreactive T lymphocytes in multiple sclerosis determined by antigen-induced secretion of interferon- γ . *J Clin Invest* 1990;86:981-5.
- 12 Link H, Sun J-B, Wang Z, Xu Z, Löve A, Fredriksson S, Olsson T. Virus-reactive and autoreactive T cells are accumulated in cerebrospinal fluid in multiple sclerosis. *J Neuroimmunol* 1992;38:63-74.
- 13 Sun J, Link H, Xiao BG, Diener P, Andersson G, Ekre HP, et al. T and B cell responses to myelin-oligodendrocyte glycoprotein in multiple sclerosis. *J Immunol* 1991;146:1490-5.
- 14 Olsson T, Sun J, Hillert J, Höjberg B, Ekre HP, Andersson G, et al. Increased numbers of T cells recognizing multiple myelin basic protein epitopes in multiple sclerosis. *Eur J Immunol* 1992;22:1083-7.
- 15 Kostulas V, Link H, Levfert AK. Oligoclonal IgG bands in cerebrospinal fluid: Principles for demonstration and interpretation based on finding in 1,114 neurological patients. *Arch Neurol* 1987;44:1041-4.
- 16 Schumacher GA, Beebe G, Kibler R, Kurland LT, Kurtzke JF, McDowell F, et al. Problems of experimental trials of therapy in multiple sclerosis: report by the panel on the evaluation of experimental trials of therapy in multiple sclerosis. *Ann NY Acad Sci* 1965;122: 552-568.
- 17 Deibler GE, Martensson RE, Kies MV. Large scale preparation of myelin basic protein from central nervous tissue of several mammalian species. *Prep Biochem* 1972; 2:139-65.
- 18 Hampson DR, Poduslo SE. Purification of proteolipid protein and production of specific antiserum. *J Immunol* 1986;136:117-29.
- 19 Quarles R, Barbarash G, Filewicz D, McIntyre L. Purification and partial characterization of the myelin associated glycoprotein from adult rat brain. *Biochem Biophys Acta* 1983;757:140-3.
- 20 Andersson G, Ekre HP, Alm G, Perlmann P. Monoclonal IFN- γ . Adaption for determination in human serum and plasma. *J Immunol Methods* 1989;125:89-96.
- 21 Kabilan L, Andersson G, Lolli F, Ekre HP, Olsson T, Troye-Blomberg M. Detection of intracellular expression and secretion of interferon-gamma at the single cell level after activation of human T cells with tetanus toxoid in vitro. *Eur J Immunol* 1990;20:1085-9.
- 22 Leiter EH, Christianson GJ, Serreze DVT, Ting AT, Worthen SM. MHC induction by interferon γ on cultured mouse pancreatic B cells and macrophages. *J Exp Med* 1989;170:1243-62.
- 23 Wick G, Brezinischek HP, Hála K, Dietrich H, Wolf H, Kroemer G. The obese strain of chickens: An animal model with spontaneous autoimmune thyroiditis. *Adv Immunol* 1989;47:433-500.
- 24 Massa PT, ter Meulen V, Fontana A. Hyperinducibility of Ia antigen on astrocytes correlates with strain-specific susceptibility to experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* 1987;84:4219-23.
- 25 Panitch HS, Hirsch RL, Schindler J, Johnson KP. Treatment of multiple sclerosis with gamma-interferon: Exacerbation associated with activation of the immune system. *Neurology* 1987;37:1097-102.
- 26 Mosmann TR, Coffman RL. Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv Immunol* 1989;46:111-47.
- 27 Wang WZ, Olsson T, Kostulas V, Höjberg B, Ekre HP, Link H. Myelin antigen reactive T cells in cerebrovascular diseases. *Clin Exp Immunol* 1992;88:157-62.
- 28 Mustafa MI, Diener P, Höjberg B, van der Meide P, Olsson T. T cell immunity and interferon-gamma secretion during experimental allergic encephalomyelitis in Lewis rats. *J Neuroimmunol* 1991;31:165-77.
- 29 Lehmann PV, Forsthuber T, Miller A, Sercarz EE. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. *Nature* 1992;358:155-7.