Immunoglobulin M in the cerebrospinal fluid: an indicator of recent immunological stimulation

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SUMMARY Using a sensitive new method for detecting oligoclonal immunoglobulin M (IgM) in unconcentrated cerebrospinal fluid (CSF), the intrathecal synthesis of IgM was correlated with that of free light chains in patients with multiple sclerosis, infections of the central nervous system and other neurological conditions. A strong correlation was found between the presence of oligoclonal IgM and that of free light chains in the CSF from patients with multiple sclerosis and infections of the central nervous system. No oligoclonal IgM response was observed in patients with non-inflammatory or non-infectious neurological conditions. This implies that oligoclonal IgM has the same diagnostic significance as free light chains.

Electrophoretically restricted immunoglobulins (Ig) commonly occur in the cerebrospinal fluid (CSF) of patients with inflammatory and infectious diseases of the central nervous system (CNS) in the form of oligoclonal bands where each band represents the product of a single B-lymphocyte clone.

Another immunological parameter is CSF free light chains. Cohen and Bannister¹ established that lymphocytic cells obtained from the CSF of a patient with multiple sclerosis do synthesise a relatively high amount of kappa light chains. Since that observation, CSF examination for light chains has become a useful diagnostic test in inflammatory diseases of the CNS²⁻⁴ as they were regarded as further evidence of intrathecal synthesis of Igs, especially during intense immunogenic stimulation.⁵

The oligoclonal nature of free light chains is well established.⁶⁻⁹ Hitherto, in almost all reports their detection in CSF is correlated with that of oligoclonal IgG. But if free light chains are related to an antigenic challenge within the CNS, their correlation with oligoclonal IgM is more significant. Immunoglobulin M is responsible for the primary immune response and its detection in CSF indicates the presence of committed lymphocyte clones elaborating an immune response against a recent offending antigen or a persistent antigenic challenge.

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Despite the potential importance of CSF oligoclonal IgM, the pace of progress in this field has been hampered by methodological difficulties in detecting the very low amounts of IgM that are usually present in CSF. We have applied a new sensitive method¹⁰ of detecting qualitative alterations of CSF IgM to study the relationship between intrathecally synthesised IgM and free light chains in patients with various neurological disorders. The present paper reports these findings and discusses the potential diagnostic importance of oligoclonal IgM in neurological diseases.

Patients

A total of 399 patients with various CNS diseases were studied (table 1). They were selected according to the provisional diagnosis at the time of their admission to hospital. Paired CSF and serum specimens were available from all of them. All specimens were examined within a week of the lumbar puncture and blind to diagnosis.

After the completion of the study, we retrospectively gathered reliable clinical data on all patients from the admission notes and/or discharge summaries. The largest patient group was that with multiple sclerosis, the majority of whom were considered to be either "clinically definite" or "early probable or latent" cases.¹¹ Only nine patients were "suspected" cases of multiple sclerosis.

Patients with CNS infections include 22 with encephalitis, 21 with meningitis, six with treated neurosyphilis, three cases of Lyme disease, and five with tropical spastic paraparesis. Inflammatory CNS diseases comprised nine patients with neurosarcoid, four CNS lupus, three Behçet's encephalitis, five with acute disseminated encephalomyelitis, three with

Table 1 Number of patients with positive oligoclonal IgM, free kappa, and free lambda light chains in the CSF, listed according to their diagnostic categories

Diagnostic categories		Oligo-clonal IgM	Free light chains	
			Kappa	Lambda
Normal control	n = 20	0	0	0
Multiple sclerosis*:				
(a) Definite or early probable/latent	n = 143	83	34	78
(b) Suspected	n = 9	4	1	1
Active neurosyphilis*	n = 12	12	5	7
CNS infections†	n = 57	36	5	13
Acute infective polyneuritis	n = 12	5	0	1
Inflammatory CNS diseases	n = 31	17	2	2
Degenerative CNS diseases	n = 23	0	1	1
Neuropathies	n = 33	0	0	0
CNS tumours	n = 13	2	0	1
Cerebrovascular diseases	n = 10	0	1	0
Spinal cord & root compression	n = 36	0	0	0
Total	n = 399	159	49	104

*Pathological values differ significantly from control $p < 0.001$.

†Pathological values differ significantly from control $p < 0.01$.

Tolosa-Hunt syndrome, and seven with various acute demyelinating conditions. Degenerative CNS diseases included Alzheimer disease and other dementias, Parkinsonism, and motor neuron disease.

Methods

The method of glutaraldehyde enhanced immunofixation¹⁰ was utilised to detect oligoclonal IgM in unconcentrated CSF. In brief, CSF and homologous serum proteins were separated by agarose electrophoresis, transferred to nitrocellulose membrane which was activated by 1% glutaraldehyde then immunofixed for IgM by a F(ab')₂ fragment of anti-IgM antiserum. The method of replicate immunoblotting technique¹² was used to detect free light chains in the CSF.

Results

Of the 399 patients examined, 159 (40%) were positive for CSF oligoclonal IgM, 104 (26%) had bands of free lambda light chains and 49 (12%) had free kappa bands in the CSF (table 1). The number of free kappa light chains detected in the CSF varied from one to six individual separate bands while free lambda chains ranged from one to nine bands. A more detailed

Fig 1 Plot of the number of free kappa and lambda light chains (ordinates) versus intrathecal synthesis of IgM (abscissas) in patients with multiple sclerosis ($n = 152$). The number of patients with more than 2 free light chains correlates significantly well with the presence of oligoclonal IgM ($p < 0.0001$ for free kappa, $p < 0.001$ for free lambda). Filled circles represent patients with clinically definite or early probable or latent multiple sclerosis ($n = 143$), open circles represent patients with suspected multiple sclerosis ($n = 9$).

Fig 2 Plot of numbers of free kappa and free lambda light chains (ordinates) versus intrathecal synthesis of IgM (abscissas) in patients with various CNS infections. Filled squares represent patients with encephalitis ($n = 22$), open squares represent patients with meningitis ($n = 21$), filled triangles represent patients with active neurosyphilis ($n = 12$), while open triangles represent patients adequately treated for syphilis. Filled circles represent patients with other CNS infections. Taking the entire group, the number of patients with more than 2 free light chains correlates significantly well with the presence of oligoclonal IgM in the CSF ($p < 0.005$ for free kappa, $p < 0.01$ for free lambda).

correlation between oligoclonal IgM and various neurological diseases is reported elsewhere.¹³

Of the 143 patients with definite or early probable or latent multiple sclerosis, 83 (58%) had CSF oligoclonal IgM. Of those 83 patients, 30 (36%) also had free kappa light chains (fig 1), while only three patients (5%) of the 60 patients who had no oligoclonal IgM in the CSF had free kappa light chains. This difference was very significant ($p < 0.0001$). The same difference was applicable to the frequency of occurrence of free lambda light chains: of the 83 patients who were positive for CSF oligoclonal IgM, 56 (67%) had free lambda light chains (fig 1), but only 19 (32%) of the 60 patients who were negative for oligoclonal IgM showed free lambda chains. The difference was also significant ($p < 0.001$). Of the nine patients with "suspected multiple sclerosis", one had five free kappa and nine free lambda light chains in the CSF.

As can be seen from fig 1, patients with multiple sclerosis were divisible into two main groups as far as the intrathecal production of IgM was concerned. In general, the group of patients who were negative for oligoclonal IgM showed little, if any, amount of free

light chains in the CSF. In numerical terms, no patient from this group had more than two free light chains. Conversely, patients who were positive for oligoclonal IgM did show greater amounts of CSF free light chains: they usually had more than two free light chains (up to 6 kappa and/or 9 lambda). This difference in the number of the free light chains between the two groups of multiple sclerosis patients is significant ($p < 0.005$).

The distribution of free light chains in CNS infections held the same relationship to CSF oligoclonal IgM bands as that seen in multiple sclerosis patients (fig 2). Of the 48 patients positive for oligoclonal IgM, 10 (21%) had CSF free kappa light chains, while no patient negative for oligoclonal IgM had free kappa chains. This difference was significant ($p < 0.005$). Similarly, 19 (40%) of the 48 patients positive for oligoclonal IgM had free lambda light chains, while only one patient, a penicillin-treated neurosyphilitic, of the 21 patients negative for oligoclonal IgM had two free lambda chains. The association between oligoclonal IgM and free lambda light chains was also significant ($p < 0.01$).

Table 2 Correlation between free light chains and oligoclonal IgM in the CSF from patients with inflammatory CNS disorders, other than multiple sclerosis

Oligoclonal IgM	Free kappa*		Free lambda†	
	Present	Absent	Present	Absent
Positive	2	15	1	16
Negative	0	14	2	12

Total number of patients = 31

*p = 0.276

†p = 0.753

There was no statistically significant correlation between the presence of CSF free light chains and oligoclonal IgM bands in inflammatory CNS diseases (table 2).

Of the 115 patients grouped under other diagnostic categories listed in table 1, only two (2%) had oligoclonal IgM in the CSF; both were cases of CNS tumours. One patient, a case of CNS lymphoma, also had four CSF free lambda light chains. Free CSF light chains were detected in another two patients: one was a case of motor neuron disease who had four free kappa and five free lambda light chains, the other was a middle-aged male with cerebellar infarction who showed five free kappa light chains in CSF. Neither had CSF oligoclonal IgM.

Discussion

The present study provides good evidence that intrathecal production of IgM in some patients with multiple sclerosis and others with CNS infections follows the same trend as that of free light chains production. Whether the concomitant presence of oligoclonal IgM and free light chains in the CSF in such diseases indicates a response to a similar causative agent(s) remains to be confirmed.

Shapiro¹⁴ reported that cell suspensions of hyperimmunised rabbit lymph nodes synthesized and secreted an excess of free light chains. Furthermore, Esiri¹⁵ reported the presence of IgM-containing cells in the CNS in acute phase of poliomyelitis, probably representing an acute immune response to the invading virus. This was accompanied by an abundance of cells that contained more light than heavy chains. In contrast, no IgM-containing cells were detected in the convalescent stage, and the number of light chain-containing cells was more or less equal to that of cells containing heavy chains. Therefore, the detection of oligoclonal IgM and free light chains in CSF of patients with CNS infections may be due to a strong reaction similar to that of the hyperimmunised rabbit.

Some patients with multiple sclerosis showed similar response to the one already mentioned. Esiri¹⁶

detected a relatively high percentage of IgM in a demyelinated plaque from necropsy material of a patient with multiple sclerosis. There was also an excess of light chain-containing cells in the plaques of most patients examined (N = 20), implying that an antigenic stimulus to B-cell proliferation may reside in plaques, particularly recent ones. Although Esiri described the presence of IgM in plaque tissue as of doubtful significance, it probably represents an immunologic response akin to that of the hyperimmunised rabbit.

Vakaet and Thompson¹⁷ reported that the presence of more than two free light chains in the CSF of patients with multiple sclerosis points to a recent relapse. They also showed an inverse relationship between the number of free light chains and the duration of the disease. More recently, Bracco and collaborators¹⁸ reported that all multiple sclerosis patients with oligoclonal free light chain bands (two or more bands in CSF) were patients with quite recent clinical exacerbation not treated with immuno-suppressive drugs. In the present study no patient who was oligoclonal IgM-negative had more than two free light chains in the CSF. Therefore, detection of oligoclonal IgM in multiple sclerosis patients may amount to the presence of intense immunologic stimulation.

The detection of free kappa light chains in the CSF from patients with motor neuron disease has been described before.¹⁹ The patient we examined had free kappa and lambda light chains but oligoclonal IgM was absent in the CSF. Therefore, no conclusions could be drawn as to the significance of the free light chains in this condition. The same applies to the patient with cerebellar infarction.

The occurrence of CSF oligoclonal IgM bands in the patients with pontine tumour (see results) was expected, since it has been reported to occur in lymphoproliferative disorders.²⁰ In fact, the detection of free kappa light chains in the CSF also has certain implications. By analogy with the frequent occurrence of free light chains in patients with lymphoproliferative disorders, the presence of free light chains is a point in favour of recurrence.²

Conclusions

We conclude that oligoclonal IgM bands in the CSF have the same diagnostic significance as free light chains. Both represent the activity of an intensely stimulated mature B-lymphocyte population, and therefore may reflect the intensity of the underlying immune response. The presence of oligoclonal IgM in the CSF may be an accurate index of an ongoing intrathecal humoral immune response. Recent work^{18,21} showed that free light chains may be present in CSF from multiple sclerosis patients devoid of

detectable oligoclonal IgG bands, probably indicating an early immune response. The presence of oligoclonal IgM in such cases makes this presumption even more plausible.

The present study shows that the principal practical significance of oligoclonal IgM is its potential diagnostic value in infectious or inflammatory CNS diseases, particularly multiple sclerosis. In contrast, most IgG assays lack this specificity. For example, oligoclonal IgG bands have been observed in diseases with no apparent inflammatory or infectious causes.^{22,23} Even free light chains may show non-specificity, as when they are detected in cerebrovascular diseases or motor neuron disease.

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